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LINKAGE RELATIONS OF FACTORS FOR RESISTANCE TO MILDEW IN BARLEY

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Received May 20, 1944

INTRODUCTION

THE determination of linkage relations of the seven genes for resistance of barley to mildew, *Erysiphe graminis*, which have been discovered at this station is an important part of the study of the genetics of resistance to this disease. The goldfoil factor has been shown to belong to linkage group IV with a crossover value of 18.77 per cent with hooded and 22.58 per cent with the blue aleurone factor in that group (BRIGGS and STANFORD 1943).

The Psaknon gene, Ml_p, for resistance was first identified (STANFORD and BRIGGS 1940) in the Psaknon variety where it occurs alone. It was also found along with the Hanna factor, Ml_h, and a new recessive gene, named the Duplex gene, ml_d, in the Duplex variety. In the cross of Duplex×Atlas there was a deficiency of F3 progenies which were segregating in the ratio of 1 resistant to 3 susceptible, which, it was pointed out at that time, would result from linkage between the Duplex gene and either one of the other two factors present. Obviously, its linkage relations could not be determined from such a complex hybrid. Selection 175 from the above hybrid carries the Duplex gene only. From the appropriate crosses with Selection 175 the linkage relations for the Duplex gene have been determined. Data also are available for the Psaknon gene.

MATERIALS AND METHODS

Crosses were made between Selection 175 and the two mildew resistant varieties Hanna and Psaknon in order to determine the linkage relations of the three genes for mildew resistance. Crosses of Selection 175, Psaknon and Hanna also were made with ten linkage tester varieties which carried genes known to be present in one or more of the seven chromosome groups in barley. Several of these crosses were sampled for mildew resistance until the linkages sought were located. Then as large a population of the critical crosses were grown as seed were available.

As in previous experiments, the mildew tests were made in the greenhouse with race 3 of the fungus. Mildew classifications were based on the reaction of F_2 plants which were grown in greenhouse benches. The plants were inoculated in the three leaf stage by dusting with spores from diseased plants grown for that purpose.

Every twentieth row was seeded to susceptible Atlas as a check. Also adequate checks of the parents of the several crosses were included. High levels of infection were obtained. Therefore no difficulty was encountered in classifying the hybrids for resistance versus susceptibility to mildew.

EXPERIMENTAL RESULTS

As indicated earlier, STANFORD and BRIGGS (1940) pointed out that the data from Duplex×Atlas suggested a linkage between the Duplex gene and either the Hanna or the Psaknon gene for resistance to mildew. There were only four F_3 progenies segregating in the ratio of 1 resistance: 3 susceptible plants as compared with eight susceptible F_3 progenies. Since the cross was segregating for three factor pairs, it could not be proved that these were significant deviations even though there was a total of 289 F_3 progenies. In any case, it would not have been possible to determine which one of the two genes was linked with the Duplex gene. Data from the crosses necessary to establish the above points are reported in table 1.

TABLE 1

The F_2 segregation of resistance to mildew for the crosses named with expected numbers based on independence and on linkage where it is indicated.

CROSS	F_1 GENOTYPE	OBSERVED NUMBER F_2 SEGREGATIONS		EXPECTED NUMBER FOR INDEPENDENCE		P VALUE	LINKAGE INDICATED	EXPECTED NUMBER FOR LINKAGE INDICATED	
		RES.	SUSC.	RES.	SUSC.			RES.	SUSC.
Sel. 175×Atlas	$ml_d\,Ml_d$	118	335	113.3	339.7	>0.5			
Sel. 175×Hanna	$Ml_d\,ml_d\,Ml_h\,ml_h$	302	75	306.3	70.7	>0.5			
Sel. 175×Psaknon	$Ml_d\,ml_d\,Ml_p\,ml_p$	1316	107	1156.2	266.8	small	16.38	1316	107

A small F_2 population from Selection 175×Atlas was grown in order to definitely check the constitution of this selection. It will be seen that it segregated 1 resistant:3 susceptible plants as expected.

In the cross with Hanna the segregation was 13:3, indicating independence for the Ml_h and ml_d factors. However, there was a marked departure from the 13:3 ratio in Selection 175×Psaknon, indicating linkage between these two factor pairs, which was calculated to be 16.38 per cent. The number of plants expected on the basis of the above linkage is in complete agreement with those observed, which follows because there are only two classes of plants.

If this crossover value is applied to the data from Duplex×Atlas (STANFORD and BRIGGS 1940) mentioned above, 4.9 segregating and 12.6 susceptible rows are expected as compared with 4 and 8 obtained.

Small populations from crosses of Psaknon with Colsess IV, *Hordeum nudideficiens*, and Brachytic were sampled for linkage before it was discovered that the Psaknon factor pair was linked with the normal versus albino seedling, *At at*, factor pair carried by Trebi I. In the three crosses mentioned first mildew resistance versus susceptibility was independent of the factor pairs,

hooded versus normal lemma, normal versus xantha seedlings, non-six-row versus six-row spike, hulled versus naked caryopsis and normal versus Brachytic plant stature which belong to linkage groups IV, VI, I, III, and VII respectively (ROBERTSON, et al. 1941).

As soon as it was discovered that the Psaknon gene pair was linked with the *At at* gene pair, all the seed from families segregating for both gene pairs were planted. Likewise, all families from Selection 175×Trebi I which were segregating for normal versus albino seedlings were planted. These data are given in table 2.

In the Psaknon times Trebi I cross the probability of the two gene pairs under consideration being independent is very small. The crossover value

TABLE 2

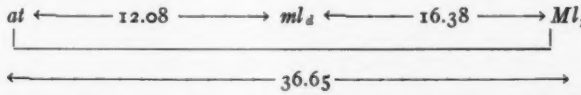
The F_2 segregation of the characters indicated for the crosses named with expected numbers based on independence and on the linkage indicated.

CROSS	F_1 GENOTYPE	OBSERVED F_2 SEGREGATION			EXPECTED FOR INDEPENDENCE			P VALUE	LINKAGE INDICATED	EXPECTED FOR LINKAGE INDICATED			P VALUE
		NORMAL RES.	NORMAL SUSC.	ALBINO	NORMAL RES.	NORMAL SUSC.	ALBINO			NORMAL RES.	NORMAL SUSC.	ALBINO	
Psaknon ×Trebi I	$\frac{Ml_p\ At}{ml_p\ at}$	1845	460	701	1609.9	563.6	751.5	small	36.65	1804.6	449.9	751.5	>.02
Sel. 175 ×Trebi I	$\frac{ml_D\ At}{Ml_D\ at}$	495	1012	519	397.9	1139.5	506.5	small	12.08	499.1	1020.4	506.5	>.4

indicated is 36.65 per cent. The expected numbers based on that value give a probability between .02 and .05 which is still low. This low P value is largely due to the fact that there was a deficiency of albino plants which must have been a chance deviation.

A recombination percentage of 12.08 is indicated in the cross of Selection 175 with Treti I. The χ^2 for the observed versus the numbers expected on the basis of the above linkage gives a P value greater than 0.4.

It is clear from the above data that the Psaknon, Ml_p, and the Duplex, ml_d, genes for resistance to mildew are linked with each other and in turn are linked with the normal versus albino, *At at*, factor pair which belong in linkage group II (ROBERTSON, et. al. 1941). The following order is indicated.



The two gene pairs for resistance to mildew makes a total of five gene pairs positively identified as belonging to linkage group II which has been represented by a smaller number than any other group. In addition to normal versus albino seedlings, those previously reported were black versus white and normal versus third outer glume.

SUMMARY

From a cross between Psaknon, which carries the Psaknon factor Ml_p, for resistance to mildew and Selection 175 which is a selection from Duplex ×Atlas and carries the Duplex factor, ml_d, for resistance to this disease, it was found that these two genes for resistance were linked with a recombination value of 16.38 per cent.

These genes were found to be linked with the factor pair, normal versus albino (*At at*) which places them in linkage group II. The crossover percentages were 12.08 for the Duplex and 36.65 for the Psaknon genes respectively.

The gene order indicated is *at*, ml_d, Ml_p.

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BACTERIOPHAGE-RESISTANT MUTANTS IN ESCHERICHIA COLI

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Received August 8, 1944

BACTERIA are very promising as materials for studies of mutability, because of their great speed of reproduction and because of the opportunity they present for working with populations that comprise enormous numbers of individuals. Changes in bacterial cultures which make the bacteria resistant toward certain specific bacteriophage strains have been known for a long time and have been assumed by several workers to be due to mutations (GRATIA 1921; BURNET 1929; and others). This assumption has recently been confirmed in an important study by LURIA and DELBRÜCK (1943). The experimental method used by these investigators consists of determining how many resistant bacteria are present in small cultures of sensitive bacteria, especially when the size of cultures is kept so small that only a few resistants are found, on the average, in each culture. Under these conditions some cultures are still found to contain a comparatively large number of resistants. This indicates that such bacteria probably belong to a single clone stemming from an ancestor which mutated to bacteriophage resistance earlier in the growth of the culture.

Changes to bacteriophage resistance have been known to be fairly specific (BAIL 1923; BURNET 1929), inasmuch as each observed change usually involves resistance to only some of the bacteriophage strains capable of attacking the original strain of bacteria. The problem of resistance in bacteria therefore offers an unusual opportunity for a quantitative study of the occurrence of specific types of mutants, which outweighs the disadvantage of not being able to check the behavior of the mutants during a sexual process.

The purpose of the present study is to extend the investigation of LURIA and DELBRÜCK to cover resistance to several bacteriophage strains active on the same line of *Escherichia coli*. In particular, we wished to determine how bacteria attain simultaneous (or "multiple") resistance to various bacteriophage strains—that is, whether or not this resistance is due to separate, independent mutational steps.

MATERIALS AND METHODS

The materials used in our experiments consisted of the same bacterial strain —*E. coli* **B**—previously used by LURIA and DELBRÜCK (1943), of seven phage strains active on **B**, and of various strains of bacteria, derived by mutation from **B**, which were resistant to one or more of the phages. The phage strains were indicated as type 1 to type 7 (T1 to T7). T1 and T2, with which DR. LURIA supplied us, are the alpha and gamma strains of DELBRÜCK and LURIA (1942) and are identical with the P28 and PC strains of DR. J. BRONFENBRENNER (KALMANSON and BRONFENBRENNER 1939); T3, T4, T5, and T6 were isolated from a mixture of phages supplied by DR. TONY L. RAKIETEN; T7 was

isolated from the standard anti-coli-phage mixture prepared by DR. W. J. MACNEAL. Plaques produced by these phages were studied by plating a mixture consisting of phage and of bacteria obtained by washing a 24-hour slant with broth. For all our plates we used a nutrient agar, prepared by dissolving in the standard Difco nutrient broth 1.5 per cent of flaked agar and 0.5 per cent of NaCl. To minimize air contamination during manipulation of the plate, one drop of a saturated aqueous gentian-violet solution was added to each liter of the medium (BURNET 1929). We found it helpful against contamination, and particularly against condensation of the moisture within a Petri dish, to place filter paper on the inside of the Petri-dish cover. Cultures were incubated at 37°C.

In our experimental conditions T1 produces large plaques about 0.5 to 2 mm in diameter, visible after five hours of incubation, which after about 18 hours of incubation develop a large halo. Plaques of T2 are small, from 0.2 to 1.0 mm in diameter, without a halo and visible after about 18 hours of incubation. However, when T2 is grown on very soft agar, by the technique developed by HERSHEY, KALMANSON and BRONFENBRENNER (1943), an appreciable proportion of plaques develop small halos. T3 plaques are large, 1 to 2.5 mm in diameter; they become visible after five hours of incubation and later develop a large halo with a light ring around the central portion of the plaque. T4 plaques are small, 0.3 to 1.0 mm in diameter, and develop after about five hours of incubation; a small proportion of them show a small halo. Subsequently it was found that plaques without halos and those with halos breed true, and two lines of T4 were isolated—namely, T4a, which does not have a halo, and T4b, which has a halo. In all resistance tests made with these two strains their behavior was identical. T5 plaques are of medium size, about 0.5 to 1 mm in diameter; they show up after about five hours, and later develop a narrow halo. T6 has small plaques, similar to T2, but they develop early, after about five hours of incubation. T7 in appearance is very similar to T3—that is, it has large plaques with a large halo containing a ring. Plaques appear after five hours of incubation. Photographs of plaques of seven phages are reproduced in Plate 1, figures 1 to 7.

In this work we wanted to determine the number of mutants resistant to a particular phage that are present in a series of small, similar cultures of a bacterial strain sensitive to that phage. A suspension of sensitive bacteria was prepared in nutrient broth, containing about 1000 bacteria per cc. The desired amount, usually 0.3 cc, of this suspension was sucked up into the center of each of a large number of sterile 1-cc pipettes, after which the pipettes were stored in a sterile metal holder and incubated for about 24 hours. At the end of this period the concentration of bacteria in several sample pipettes was assayed for each experiment by the method of colony counts. This concentration was usually about 10^8 per cc—that is, the average number of bacteria per pipette was of the order of magnitude of 30 million. The contents of each remaining pipette were then spread on a nutrient-agar plate which had previously been coated with an excess of phage. At least 0.1 cc of phage suspension, of a titer of

about 10^9, was used on each plate. The plates were then incubated for 24 hours, and in later experiments for 48 hours, prior to observation and classification of the colonies of resistant bacteria that had developed.

To compare the numbers of mutants resistant to different phages and various combinations of phage mixtures, which were present in a particular culture of a sensitive bacterial strain, equal samples of the bacterial culture were mixed with excess amounts of one or more phages and spread on different plates.

Bacterial strains derived from colonies grown in the presence of excess phage were designated, following the earlier usage of BURNET and MCKIE (1936), by the symbol of the sensitive strain, a crossbar, and the number of the phage used in the experiment. Thus, resistant strains obtained as a secondary growth after plating the strain **B** on a Petri dish coated with phage T1 were designated as **B**/1. If **B**/1 was later plated on a Petri dish coated with a mixture of T_3 and T_4, the resulting colonies were designated as **B**/1/(3, 4).

In order to establish a representative set of mutant strains for purposes of further analysis, we had somehow to deal with the still unsolved problem of "lysogenicity." This term is used in connection with phenomena of apparently incomplete resistance, in which phage and bacteria multiply at the same time and in the same culture. The standard method of establishing a mutant strain was to inoculate broth with material from a bacterial colony that had grown on agar in the presence of excess phage. By this procedure the inoculum contained some phage as well as bacteria. The development of the culture appeared to be connected with the morphological appearance of the original colony as follows:

(a) Round colonies with the same structure as colonies of the parent strain give rise to normal growth in broth. The presence of some phage in successive cultures of the strains thus established does not make itself felt in tests of resistance to other phage types. The phage itself can be easily eliminated from the culture by repeated isolation of single colonies. This was done whenever a resistant strain was used in experiments other than routine cross-resistance tests.

(b) "Nibbled" colonies, with irregular edges and normal structure except on "chewed-up" sectors, which have a watery-translucent consistency, give rise to good growth in broth. The presence of phage in successive cultures of the strains thus established becomes evident, however, whenever a thick suspension of bacteria is plated onto agar: scattered plaques of the phage appear on the confluent bacterial growth. Phage assays indicate that the phage multiplies at the same time as the bacteria, and that only a small fraction of the phage particles present in the bacterial culture gives rise to plaques when the culture is plated on agar. Repeated isolation of single colonies from such cultures leads to eventual elimination of the phage, after which the bacterial strain does not appear to differ from the strains discussed under (a).

(c) "Thin" colonies, with watery-translucent structure throughout, do not give rise to good growth in broth. Attempts to culture them in broth yield instead a substantial concentration of phage. Repeated streaks on agar derived

from such colonies develop very slowly, but generally result in eventual elimination of the phage and in the establishment of a strain that does not appear to differ from the strains discussed under (a).

Tests of resistance of the bacterial strains to various phage strains were made by the cross-streaking method. On a nutrient agar plate, suspensions of bacteria and of phage were streaked with a large loop at right angles; and the growth, or lack of growth, of bacteria at the intersection of the streaks was observed. A bacterial strain was classified as sensitive to a phage if it showed lysis at the intersection of streaks. In a few instances, a variation in the degree of lysis was observed, but no attempt was made to record it.

RESULTS

Types of mutants.—Small broth cultures (0.3 cc) of the sensitive strain **B** regularly yielded mutants resistant to phages T1, T3, T4, T5, T6, and T7,

TABLE 1

*Cross-resistance tests with various bacterial strains derived from **B** line.*

TYPE	NUMBER OF STRAINS TESTED	RESISTANT TO PHAGE						
		T1	T2	T3	T4	T5	T6	T7
B/1	61	all	0	1	1	53	0	0
B/3	85	1+1	1	all	all	1+1	1	49+1
B/4	42	2	2	all	all	2	2	23+2
B/5	40	all	0	0	0	all	0	0
B/6	61	1	0	0	0	1	all	0
B/7	88	2+1	1	all	all	2+1	1	all

which were designated as **B**/1, **B**/3, **B**/4, **B**/5, **B**/6, and **B**/7, respectively. The frequency of occurrence of these mutants in the cultures varied between 10^{-8} and 10^{-5} approximately of the titer of normal bacteria, being in general lowest for **B**/1 and **B**/5.

No success was obtained in trying to isolate **B**/2 mutants by the standard method of plating **B** with an excess of phage T2, even though large amounts of high-titer cultures of **B** were plated in this way. Such platings generally gave rise to a thin, irregular growth of bacteria, which did not prove to be resistant. This growth is thought to be related to the peculiar slowness of action of T2 on **B** on agar, which is also apparent in the tardy appearance of T2 plaques. The observed growth may obscure the occurrence of a very small number of mutants truly resistant to T2.

Large numbers of strains of **B**/1, **B**/3, **B**/4, **B**/5, **B**/6, and **B**/7 were tested for resistance to all of our phage types. To make certain that the strains tested had stemmed from independent mutations, they were in the majority of instances derived from platings of separate cultures of **B**. Only occasionally were two strains derived from one plating, and that only when the plating of one

culture of **B** with excess phage showed two families of colonies clearly different in their morphology. In such cases one strain was established from each family. The numbers of strains that were tested and found to be resistant to the various phages are shown in table 1.

The strains entered in the table in italics proved to be resistant to all seven phages, including T2. Although the colony appearance is similar to that of the **B** strain, we do not feel certain that these colonies were not derived from contaminations, because they were resistant to T2, which is an exceptional condition, and because we did not make special identification tests. If these strains were not contaminants, they would seem to be mutants belonging to a different class than the other strains listed in the table.

In our case, as in others previously described (BURNET and MCKIE 1936), the phages may be grouped into cross-resistance groups. In the T1, T5 group all **B**/5 strains are also resistant to T1, while about 90 per cent of **B**/1 are also resistant to T5. This indicates the existence of two distinct types of mutants, one of which is resistant to T1 only, and the other to both T1 and T5. The first type is designated as **B**/1; the second should be designated either as **B**/1, 5 or **B**/5, 1, according to whether it was detected by plating **B** on T1 or on T5. Thus strains that are detected as **B**/1 are a mixture of **B**/1 and **B**/1, 5, while all strains thus far detected as **B**/5 are actually **B**/5, 1. No difference has been detected between strains designated as **B**/1, 5 and **B**/5, 1. The indication **B**/(1, 5) is used when the distinction is immaterial or when no distinction can be made.

As an additional check on the grouping of phages in resistance groups, a few experiments were done in which equal samples from one culture of **B** were plated on T1, on T5, and on a mixture of T1 and T5. It was expected that both types of mutants, **B**/1 and **B**/(1, 5), would form colonies on plates coated with T1 and that only **B**/(1, 5) mutants would form colonies on plates coated either with T5 or with the mixture of T1 and T5. Consequently, the number of colonies on the two sets of plates with T5 or with the mixture should be approximately equal and should as a rule be smaller than the number of colonies on the first set of plates. The colony counts in four experiments were:

Plates coated with:	T1	T5	T1+T5
Culture—1	135	62	29
—2	169	154	179
—3	241	43	51
—4	332	86	62

The colony counts in different experiments are unrelated, being derived from different cultures. In these cultures the total number of bacteria was high, and therefore it is to be expected that they would contain many mutants. The proportion of **B**/1 and **B**/(1, 5) mutants may differ from culture to culture, depending on which mutation happens to occur earlier. Culture 1 appears to contain **B**/1 and **B**/(1, 5) mutants in a ratio of about 2:1 (the difference between 62 and 29 in the count of colonies on T1 and on T1+T5 plates is as-

sumed to be due to a sampling accident). Culture 2 appears to contain B/(1, 5) but no appreciable amount of B/1 mutants. Cultures 3 and 4 appear to contain B/1 and B/(1, 5) in approximately a 4:1 ratio.

Resistance to T3, T4, and T7 also is due to a system of mutants which will be indicated as B/(3, 4) and B/(3, 4, 7). Mutants detected as B/3 or B/4 are either B(3, 4) or B/(3, 4, 7), while all mutants detected as B/7 seem to be B/(3, 4, 7). Experiments to support this interpretation, made by plating B cultures on Petri dishes coated with T3, T4, T7, and their mixtures, were carried out as for the T1, T5 group, with similar results.

Mutants detected as B/6 are generally resistant to T6 only.

Thus we may separate our seven strains of phage into four groups with regard to resistance reactions. T1 and T5 fall into one group; T3, T4, and T7 fall into a second group; while T2 and T6 form two other separate groups.

Table 1 shows occasional instances of mutants resistant to phages belonging to different resistance groups. For example, among 85 mutants detected as B/3 there was one B/3, 1, 4, 5; among 61 mutants detected as B/6, one was B/6, 1, 5; and among 88 detected as B/7, two were B/7, 1, 3, 4, 5. However, these multiple-resistant mutants occur with a much lower frequency than the mutants belonging to the regular resistance groups. Such multiple-resistant mutants can be most easily detected by plating a large number of bacteria on appropriate mixtures of phages.

In the experiments just discussed, the resistant mutants originated from the B strain, which is sensitive to all our phages. Table 2 summarizes the results of another set of experiments, in which the mutants were double or multiple mutants—that is, they were obtained from an already-resistant strain derived from B in previous experiments. Tests were made with a representative sample of resistant strains, and when uniform results were obtained no effort was made to complete the tests with all available resistant mutants. It is evident from the data in table 2 that there is no difference between the behavior of the resistant strains and the behavior of B strain in regard to the yield of new resistant mutants—that is, the pattern of resistance groups is the same irrespective of whether mutants were obtained from the B strain or from a resistant strain. For example, B/1 strain gives B/1/5, B/1/(3, 4), B/1/(3, 4, 7), and B/1/6 mutants; B/6 strain gives B/6/1, B/6/(1, 5), B/6/(3, 4), and B/6/(3, 4, 7) mutants, etc. New mutations are generally superimposed on the pattern of resistance which the strain had due to previous mutation; as a rule they are not affected by this pattern nor do they affect it. Table 2 shows the occurrence of one exceptional case, in which a B/6/1/7 strain was found not to be resistant to either T3 or T4.

Mutation rates.—Luria and Delbrück (1943) stressed the fact that considerable variation is observed in proportions of resistant mutants in parallel cultures of bacteria. This variation is the natural consequence of the mutational origin of resistance, because of the clonal grouping of mutants in each culture. Mutation rate is defined as the probability of occurrence of a mutation per bacterium per bacterial generation. Two alternate methods of estimating

the mutation rate have been proposed by LURIA and DELBRÜCK and used in our work.

(1) Determination based on the percentage (p_0) of cultures which do not contain any resistant mutant. The formula yielding the mutation rate (a) per bacterial generation is:

$$a = \frac{1.6}{N} \log_{10} p_0 = \frac{\ln 2}{N} \ln p_0$$

where N is the average number of bacteria per culture. The natural logarithm of 2 appears because the mutation rate refers to one bacterial generation—

TABLE 2

Cross-resistance tests with bacterial strains derived from various resistant lines.

PARENT STRAIN	NEW RESIST-ANCE	NO. OF TESTS	RESISTANT TO PHAGE						
			T1	T2	T3	T4	T5	T6	T7
B/1	/3	25	all	0	all	all	0	0	9
	/4	20	all	0	all	all	0	0	12
	/5	22	all	0	0	0	all	0	0
	/6	23	all	1	1	1	1	all	1
	/7	22	all	0	all	all	0	0	all
B/6	/1	37	all	2	2	2	34	all	2
	/3	39	0	0	all	all	0	all	21
	/4	21	0	0	all	all	0	all	12
	/5	12	all	0	0	0	all	all	0
B/4, 3	/1	13	all	0	all	all	all	0	0
	/5	6	all	1	all	all	all	1	1
B/4, 3, 7	/1	10	all	1	all	all	7	1	all
	/5	6	all	0	all	all	all	0	all
	/6	7	0	0	all	all	0	all	all
B/6/1	/3	20	all	2	all	all	1	all	10
	/5	25	all	0	0	0	all	all	0
	/7	30	all	3	29	29	3	all	all
B/6/3, 4	/1	10	all	0	all	all	7	all	2
	/5	11	all	1	all	all	all	all	1
	/7	5	0	0	all	all	0	all	all

that is, to the time during which the population doubles in number. This method is not very reliable, unless the conditions are so arranged that p_0 is neither too small nor too large; in fact, the method cannot be used if every culture contains mutants. Since we tried to use standardized experimental conditions, this prevented us from keeping p_0 steadily within the optimum range; and in a number of cases p_0 actually fell to zero. However, this method is inherently more accurate than the alternate method, inasmuch as it is affected to a much smaller degree by systematic errors.

(2) Determination based on the average number (r) of mutants per culture.

The formula correlating r with the mutation rate a per bacterial generation is:

$$r = 1.6\ a\ N \log_{10} \frac{CaN}{.693} = \frac{aN}{\ln 2} \ln \frac{CaN}{\ln 2}$$

where C is the number of cultures in the experiment. This method is applicable to a wider range of conditions than the previous one, but depends upon several assumptions that hold only very approximately. Furthermore, the incorrectness of these assumptions has the effect of making the calculated value of the frequency of mutations greater than the real value. The most important among such assumptions, as pointed out by LURIA and DELBRÜCK, is to disregard the chance of mutations early in the growth of the cultures.

The experiments on the detection of mutants resistant to phage T_3 are listed in table 3. As was found in the case studied by LURIA and DELBRÜCK, the mutation rates computed by method (2) are generally larger than those computed by method (1). In three experiments all cultures carried mutants, and so method (1) could not be applied. The values obtained by method (1) range from 1.4×10^{-8} to 9.2×10^{-8}, a range that is not excessive in view of the limitations inherent in the method, besides those connected with accidental errors. The values obtained by method (2) range from 4.2×10^{-8} to 31.6×10^{-8}.

In view of the bias toward higher values inherent in method (2), it is probably fair to estimate that the rate of mutation to resistance to T_3 is somewhere in the middle of the range 10^{-8} to 10^{-7}. This rate is actually the sum of the rates of a number of mutations leading to the same resistance, in this case to **B**/(3, 4) and **B**/(3, 4, 7), the first of which, according to the results shown in table 1, is probably somewhat (50 per cent) greater than the second.

An important conclusion to be drawn from table 3 is that strains of bacteria already resistant to T1 or T6, or both, appear to exhibit the same mutation rate to resistance to T_3 as the original sensitive strain **B**. This indicates that mutations to different types of resistance are independent.

Series of experiments on the detection of mutants resistant to T1, T_4, T_5, T6, and T_7, respectively, were carried out to an extent comparable to the series on resistance to T_3. Table 4 is analogous to table 3 and includes all results. The rates of mutation to resistance to T1 and to T_5 are probably lower than the rate of mutation to resistance to T_3, inasmuch as they are probably smaller than 10^{-8}, which is in agreement with the determination of LURIA and DELBRÜCK for T1. On the basis of the data given in table 1, one must assume that the rate of mutation to resistance to T1, being the sum of rates of mutation to **B**/1 and to **B**/(1, 5), is actually somewhat greater than the rate of mutation to resistance to T_5, which is the same as the rate of mutation to **B**/(1, 5) only.

The rate of mutation to resistance to T_4, as expected, does not appear to be different from the rate of mutation to resistance to T_3. The rate of mutation to resistance to T_7, however, might appear from the data to be, on the whole, greater than the rate of mutation to resistance to T_3 and T_4. This would disagree with the results on the types of mutation to resistance to T_3, T_4, and T_7

presented in an earlier section, which require that resistance to T_7, being due to mutation to **B**/(3, 4, 7) only, be somewhat less than half as frequent as resistance to T_3 and T_4, which is due to mutation to either **B**/(3, 4, 7) or **B**/(3, 4). In view of the greater reliability of the results on the types of mutation, we ascribe the apparent disagreement to inaccuracy in the determination of the rate of mutation to resistance to T_7.

TABLE 3

Frequency of mutants resistant to phage T3.
(Tabulation of all experiments.)

PARENT STRAIN	No. OF CULTURES	AVERAGE NO. OF BACT. PER CULTURE	PERCENTAGE OF CULTURES WITHOUT MUTANTS	NO. OF MUTANTS PER PLATE		MUTATION RATE	
				AVER.	RANGE	FROM f	FROM r
	C	N	f	r			
B	37	$.29\times10^8$	8.1	12	0–39	6.0×10^{-8}	6.2×10^{-8}
B	25	.67 "	4	47	0–254	3.3 "	9.3 "
B	23	.54 "	26	14	0–78	1.7 "	4.3 "
B	25	.38 "	8	110	0–988	4.6 "	31.6 "
B	41	.075 "	44	2.3	0–18	7.6 "	6.4 "
B	42	.16 "	12	8	0–36	9.2 "	8.1 "
B	46	.36 "	17	10	0–93	3.4 "	4.2 "
B/1	30	.25 "	—	51	9–144	—	25.2 "
B/6	20	.6 "	30	21	0–83	1.4 "	5.3 "
B/6	20	.16 "	15	6.9	0–55	8.3 "	8.1 "
B/6/1	29	.92 "	—	133.8	16–1000+	—	16.3 "
B/1/6	30	.4 "	—	46	3–157	— "	14.0 "

The rate of mutation to resistance to T6 is not very different from the rates of mutation to resistance to T_3, T_4, or T_7, and is probably somewhere between 10^{-8} and 10^{-7}.

Correlation between resistance and the appearance of the colony.—The strain **B** of *E. coli* used in these experiments forms on our medium, in about 24 hours at 37°C, isolated round colonies measuring 2 to 4 millimeters in diameter and having a semirough surface. The surface of the colonies has a characteristic appearance.

It has been observed that resistant colonies derived by plating a broth culture of **B** on a Petri dish that has been previously spread with excess phage frequently appear different from the colonies of the parent strain. In general, it is possible to distinguish the following types:

(1) Large colonies, similar to the parent type.

TABLE 4

Frequency of mutants resistant to various types of phage.
(Tabulation of all experiments.)

PHAGE TYPE	PARENT STRAIN	NO. OF CULTURES	AVERAGE NO. OF BACT. PER CULTURE	PERCENTAGE OF CULTURES WITHOUT MUTANTS	NO. OF MUTANTS PER PLATE		MUTATION RATE	
					AVER.	RANGE	FROM f	FROM r
		C	N	f	r			
T1	B	53	$.16\times10^8$	87	.94	0–23	$.63\times10^{-8}$	1.4×10^{-8}
T1	B	53	.24 "	89	3	0–114	.33 "	2.3 "
T1	B	17	.54 "	41	11.7	0–133	1.15 "	3.9 "
T1	B	25	.72 "	48	14	0–120	.71 "	3.1 "
T1	B	19	1.2 "	32	25	0–122	.66 "	3.2 "
T1	B	38	.93 "	66	8	0–107	1.26 "	6.1 "
T1	B	45	.68 "	80	2.6	0–57	.24 "	0.8 "
T1	B	44	.38 "	71	7	0–153	.63 "	3.2 "
T1	B	45	.36 "	85	4	0–72	.31 "	2.0 "
T1	B	45	.51 "	51	3.6	0–47	.92 "	1.3 "
T1	B/3,4	30	—	70	2.0	0–19	—	—
T1	B/4,3	30	.45 "	53	10.2	0–208	1.0 "	3.8 "
T1	B/4,3,7	30	.11 "	67	16.2	0–350	2.6 "	21.8 "
T1	B/6	30	.44 "	50	2.6	0–32	1.10 "	1.3 "
T4	B	53	.22 "	11	12.7	0–80	7.0 "	8.2 "
T4	B	19	1.2 "	—	≈750	90–4000	—	≈58
T4	B	25	.4 "	12	51	0–675	3.7 "	~16.3 "
T4	B	43	.14 "	30	14	0–272	6.0 "	14.3 "
T4	B/1	30	.25 "	—	~120	2–≈2000	—	≈52 "
T4	B/6	30	.44 "	—	~68	9–800	—	≈18 "
T4	B/1/6	26	.18 "	12	5.7	0–18	8.2 "	6.1 "
T5	B	54	≈.1 "	91	1	0–45	.66 "	2.4 "
T5	B	16	1.2 "	50	3.2	0–16	.40 "	0.7 "
T5	B	17	.72 "	65	7.6	0–65	.42 "	2.1 "
T5	B	30	1.2 "	83	3.3	0–89	.11 "	0.6 "
T5	B	48	.71 "	10	~102	0–20000	2.3 "	15.5 "
T5	B	47	.41 "	81	3.6	0–73	.37 "	1.7 "
T5	B/1	44	1.4 "	2.3	33	0–289	1.9 "	2.9 "
T5	B/3,4	30	—	63	5.5	0–117	—	—
T5	B/4,3	30	.23 "	80	37.8	0–890	0.7 "	20.9 "
T5	B/4,3,7	30	.11 "	80	8.7	0–180	1.4 "	12.7 "
T6	B	30	.23 "	—	32	1–150	—	≈18.3 "
T6	B	23	.11 "	—	93	3–331	—	≈100 "
T6	B	45	.25 "	33	10	0–58	3.1 "	6.0 "
T6	B	44	.46 "	7	108	0–2000	4.0 "	≈25.0 "
T6	B	43	.26 "	5	156	0–≈1500	8 "	≈61.5 "
T6	B/1	29	≈.56 "	—	150	2–500	—	≈28.6 "
T6	B/4,3,7	31	.11 "	—	77.2	5–300	—	80.0 "

TABLE 4—*Continued*

PHAGE TYPE	PARENT STRAIN	NO. OF CULTURES	AVERAGE NO. OF BACT. PER CULTURE	PERCENTAGE OF CULTURES WITHOUT MUTANTS	NO. OF MUTANTS PER PLATE		MUTATION RATE	
					AVER.	RANGE	FROM f	FROM r
		C	N	f	r			
T_7	B	29	.45 "	—	92	20–667	—	24.4 "
T_7	B	22	.54 "	—	49	4–197	—	11.7 "
T_7	B	25	.20 "	—	42	2–212	—	28.0 "
T_7	B	45	.21 "	9	26	0–151	8 "	16.2 "
T_7	B	46	.4 "	15	37	0–378	3.3 "	11.5 "
T_7	B	43	.45 "	14	32	0–224	3.0 "	8.9 "
T_7	B/1	25	.36 "	—	193	125–300	—	≈55.5 "
T_7	B/1	30	≈.56 "	—	228	29–432	—	≈41.1 "
T_7	B/6/1	29	.75 "	—	176	33–~1200	—	≈25.3 "

(2) Small colonies, about one-half the diameter of the large ones but otherwise similar to them in shape, appearance, and rate of growth.

(3) Tiny colonies, less than one millimeter in diameter, having the markings and color of the large colonies but requiring a longer period to attain full size.

(4) "Nibbled" colonies, already referred to on page 121 as type (b), varying in size from large to tiny, with irregular edges that appear as though chewed up, and having a normal semi-rough appearance except on the chewed-up sectors, which have a watery-translucent consistency.

(5) Thin colonies, already referred to as type (c), which have a watery-translucent appearance throughout, are variable in regard to shape and size, and take longer than the large colonies to develop.

Types (1), (2), and (3) can readily be grown, either on agar or in broth, and reproduce true to form. Type (4) and type (5), as mentioned previously, after repeated transfers eventually yield colonies of either type (1), type (2), or type (3).

Analysis of the distribution of bacterial mutants (LURIA and DELBRÜCK 1943) has shown that the mutants resistant to a phage within a population of sensitive bacteria are not all of independent origin, but have been derived by multiplication from a smaller number of mutations that occurred independently during the development of that population. Therefore we expect that if a few mutants are derived from one population, plated on a single Petri dish with excess phage, they may belong to one single colony type, or to two types, or—more rarely—to three, if each colony type is characteristic of a certain clone of mutants. This was actually found and is illustrated in table 5, which is a copy of the record of one experiment, and in figures 8 and 9 on Plate 1, which are photographs of two cultures derived from two populations of bacteria plated on Petri dishes previously coated with T_4. In each figure, two types of colonies are evident; figure 8 shows large and small, and figure 9 shows large and tiny colonies.

It has been observed that certain colony types are more frequent among the

mutants resistant to one phage, while other colony types are more frequent among the mutants resistant to another phage. This shows a correlation between colonial morphology and resistance to certain phages. As a rule, among colonies of **B**/1, **B**/(1, 5), and **B**/(3, 4) a high proportion are large in size, while among **B**/6 a high proportion are small, thin, and nibbled, and among **B**/(3, 4, 7) a high proportion are tiny and thin.

The correlation between the appearance of a colony and resistance to a particular phage is especially evident in experiments where **B**/(3, 4) and **B**/(3, 4, 7) types are isolated. As has been mentioned earlier, approximately 60 per cent

TABLE 5

Distribution of various types among resistant colonies, obtained by plating cultures containing about 6×10^7 **B**/6 *bacteria on Petri dishes previously coated with T3 (Experiment 12–51).*

CULTURE	NUMBER OF RESISTANT COLONIES				
	LARGE	SMALL	TINY	NIBBLED	THIN
1	56				
2	4	43			
3	1		3		
4	12				
5	19				
6	39				
7	19				
8	6	18			
9	37	17			29
10	29	5			3
11	2				
12	5				7
13	26				
14	44				

of **B**/(3, 4) are **B**/(3, 4, 7). When the resistance of mutants obtained by plating **B** cultures on plates phaged with T3 or T4 is tested, and the colonies are classified according to their appearance, then it is seen that among the large colonies a majority (about 65 per cent) are **B**/(3, 4) and among the small colonies a majority (about 80 per cent) are **B**/(3, 4, 7) (table 6).

Comparative growth of different strains.—The determination of mutation rates from the observed frequencies of mutants rests upon the assumption that the growth rates of mutant strains do not differ appreciably from the growth rate of the original strain. LURIA and DELBRÜCK (1943) determined and compared the growth curves of the original strain **B** and of two mutant strains resistant to T1. On account of the large number of mutant strains under investigation, we resorted to the simplified but strict test of growing mutant strains in competition with **B**.

A broth culture was prepared containing a mixture of two strains—for ex-

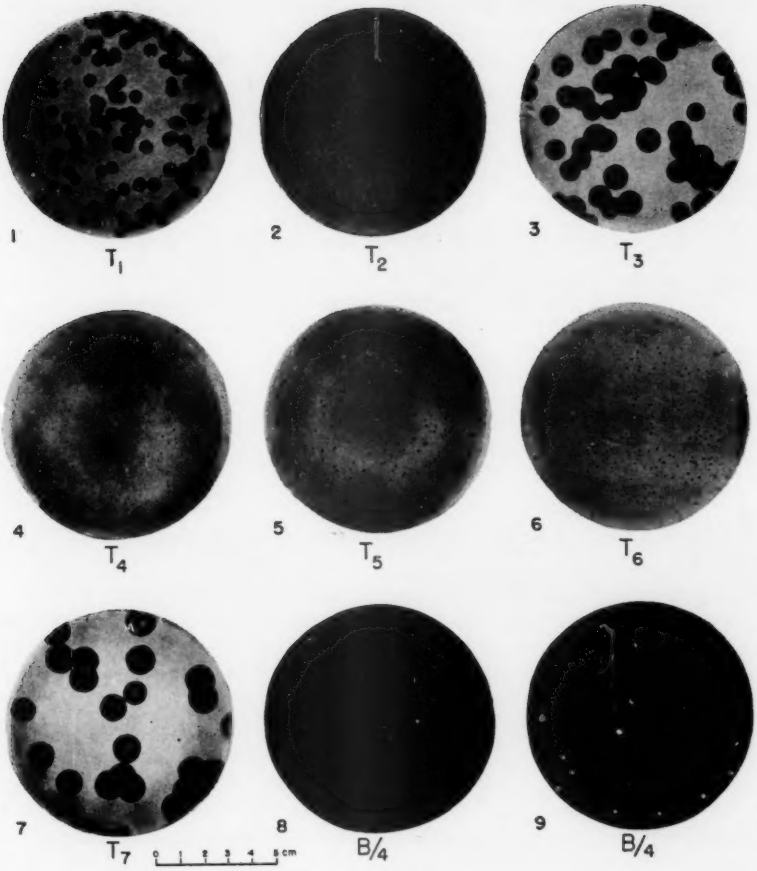

PLATE 1

FIGURES 1–7.—Photographs of plaques of phages T1 to T7 respectively. Appropriate dilutions of each phage were mixed with bacteria washed from 2½-hour slant cultures; 0.05 cc of each mixture was added to 4 cc of melted agar (0.7 per cent) at 45°C and poured into Petri dishes containing about 20 cc of solid 1.0 per cent agar medium (HERSHEY et al. 1943). Photographs were taken after about 18 hours of incubation at 37°C.

FIGURES 8 and 9.—Colonies of B/4 bacteria obtained by plating about 3×10^7 B-bacteria on agar plates previously coated with about 10^8 particles of T4 phage. Figure 8 shows large and small colonies and figure 9 shows large and tiny colonies.

ample, **B** and **B**/6. These bacteria were derived by dilution from saturated cultures and were placed in the mixture in approximately equal numbers (a few thousand per cc). The mixture was assayed by placing small samples (a) on normal agar culture medium, and (b) on agar culture medium previously spread with excess phage (in our example T6). Colony counts on (b) plates determined the concentration of **B**/6 in the culture; the difference in count of (a) and (b) determined the concentration of **B**. The ratio **B**/(a−b) is the relative concentration of the two strains. The culture was then grown to saturation (approximately 10^8 per cc) and a new determination of the relative concentration of **B** and **B**/6 was made.

TABLE 6

Resistant strains derived by using T3 and T4 phage classified according to size of the colony and resistance to T7, indicating correlation between small size and resistance to T7.

COLONY SIZE		B/(3, 4)	B/(3, 4, 7)	TOTAL
Large:	observed	86	46	132
	expected	62	70	
Small:	observed	18	72	90
	expected	42	48	
Total		104	118	222

Table 7 lists the experiments carried out and their results, including the ratio of the final and initial values of the relative concentration of the pairs of strains. This ratio is 1 when the two strains grow at the same rate, greater or smaller than 1 if the mutant grows faster or slower, respectively. Under the experimental conditions, a small difference in the rate of growth of the strains, or any accident giving a small initial advantage to one strain, may strongly affect the final value of the relative concentration. Thus a 20 per cent difference in the duration of the division cycle would change the relative concentration during the experiment by a factor as large as 10. Also a delay of one hour in the onset of multiplication of one of the strains would have by itself an effect of similar magnitude. The importance of accidental variations can be appreciated by comparing the results of repeated experiments where the same strains were used. Repeated use of the same strain is indicated by ditto marks in the first two columns in the table.

In view of this, we feel that the results shown in the table indicate a considerable uniformity in the growth rates. On the whole, growth rates for mutants are lower than for the **B** strain; but no large competitive disadvantage seems to be systematically associated with the phenomenon of phage resistance. This seems to hold even when a multiple mutant strain is examined, and even when the strain's colonies on agar are much smaller than the colonies of the original strain.

TABLE 7

Results of experiments on competitive growth.

MUTANT STRAIN		SELECTING PHAGE	INITIAL ASSAY			FINAL ASSAY			b/a
RESISTANCE	COLONY		B	MUTANT	RELATIVE CONCENTRATION (a)	B	MUTANT	RELATIVE CONCENTRATION (b)	
B/1	large	T1	371	290	.78	132	303	2.3	3.0
B/1	nibbled large	T1	493	412	.84	0	646	∞	∞
"	"	T1	99	204	2.1	48	70	1.5	.71
"	"	T1	865	357	.41	107	232	2.2	5.3
B/6	large	T6	849	109	.13	1188	48	.04	.32
B/6	nibbled large	T6	132	354	2.7	58	172	3.0	1.1
B/1, 5	large	T1	218	121	.56	0	243	∞	∞
"	"	T1	81	110	1.4	90	22	.24	.18
"	"	T1	809	298	.33	87	126	1.4	4.4
B/1, 5	large	T1	485	189	.39	28	149	5.3	14.
B/1, 5	nibbled large	T1	110	122	1.1	38	58	1.5	1.4
B/5, 1	large	T1	124	91	.73	109	40	.37	.51
B/4, 3	large	T3	153	144	.94	262	18	.07	.07
"	"	T3	218	158	.77	220	25	.11	.14
"	"	T3	157	182	1.2	228	24	.10	.09
B/3, 4	large	T3	65	69	1.1	153	19	.12	.12
B/3, 4	small	T3	487	41	.08	141	2	.01	.17
B/3, 4, 7	small	T3	32	65	2.0	18	44	2.4	1.2
"	"	T3	100	16	.16	6	3	.50	3.1
B/4, 3, 7	small	T3	676	91	.13	240	4	.02	.12
"	"	T3	101	25	.25	156	0	0	0
B/7, 3, 4	tiny	T3	58	64	1.1	136	11	.08	.07
B/6/1	large	T6	123	798	6.5	297	193	.65	.10
B/1/3, 4	large	T3	45	117	2.6	155	33	.21	.08
B/1/3, 4	medium	T3	63	70	1.1	165	2	.01	.01
B/6/1, 5	large	T1	131	226	1.7	34	84	2.5	1.4
B/6/1, 5	large	T6	168	221	1.3	99	92	.93	.71
B/6/1, 5	nibbled large	T1	121	135	1.1	64	48	.75	.67
B/5, 1/6	large	T6	500	180	.36	612	89	.15	.40
B/6/3, 4	large	T3	866	157	.18	507	12	.02	.13
B/4, 3/1, 5	large	T3	778	90	.12	431	11	.03	.22
B/4, 3/1, 5	large	T1	104	48	.46	89	5	.06	.12
B/3, 4/1, 5	medium	T1	116	86	.74	68	5	.07	.10
B/3, 4/1, 5	medium	T1	84	41	.49	115	10	.09	.18
B/4, 3/1, 5	small	T3	545	122	.22	371	9	.02	.11
"	"	T1	388	211	.54	122	6	.05	.09
B/4, 3/1,5	small	T3	602	62	.10	477	0	0	0
"	"	T1	84	70	.83	113	0	0	0
"	"	T1	116	86	.69	141	2	.01	.02
B/4, 3/1, 5	small	T3	538	78	.15	317	7	.02	.15
"	"	T1	110	65	.59	170	0	0	0
"	"	T1	149	52	.35	191	4	.02	.06
B/7, 3, 4/1, 5	small	T1	429	256	.60	487	16	.03	.06
"	"	T1	72	218	3.0	137	9	.07	.02
B/7, 3, 4/1, 5	small	T1	103	114	1.1	165	0	0	0
B/6/1/7	thin large	T1	149	345	2.3	85	121	1.4	.61
B/6/1/7, 3, 4	thin medium	T1	181	239	1.3	89	103	1.2	.92
B/6/1/7, 3, 4	thin medium	T1	226	106	.47	141	163	.87	1.9
"	"	T1	93	143	1.5	214	28	.13	.08
B/6/1/7, 3, 4	thin medium	T1	110	48	.44	177	148	.84	1.9

DISCUSSION

The evidence presented in this paper shows that we isolated eight different mutants by exposing the **B** strain of *E. coli* to the seven bacteriophage strains

(T_1–T_7) used in our experiments. Two of these mutants (**B**/1 and **B**/6) are resistant to only a single phage strain each; two others (**B**/1, 5 and **B**/3, 4) are resistant to two strains each; and the remainder (**B**/3, 4, 7, **B**/1, 3, 4, 5, **B**/6, 1, 5 and **B**/7, 1, 3, 4, 5) possess multiple resistance. Of these mutants, **B**/1, **B**/1, 5, **B**/3, 4, **B**/3, 4, 7, and **B**/6 arise more frequently than the others. The fact that some of the mutations confer on the bacteria a resistance not to a single phage strain but to several strains suggests the existence of certain relationships among the latter (BURNET and MCKIE 1936). The phages in our collection may accordingly be classified into four resistance groups. T_1 and T_5 form one group; T_3, T_4, and T_7 form another; while T_2 and T6 are the single known representatives of two further groups. Mutants that confer on the bacteria a resistance to phages belonging to different groups are rare (**B**/1, 3, 4, 5, **B**/6, 1, 5, and **B**/7, 1, 3, 4, 5).

Theoretically, the origin of bacterial lines with multiple resistances may be accounted for in two different ways. Thus a **B**/1, 3, 4, 5 strain may arise from **B** owing to a single mutational step that confers resistance to these four types of phage at once, or else mutations to **B**/1, 5 and **B**/3, 4 (which are known to arise separately) may by a coincidence occur in the same cell or cell line. Taking into account the frequencies of the mutations (see below), the probability of such coincidences is extremely low, and a great majority of mutants with multiple resistance derived directly from a sensitive strain must be regarded as representing particular one-step mutational types.

Our technique of detection of mutants has been based on obtaining colonies of bacteria resistant to certain lines of phages, on agar plates on which all the nonresistant bacteria are destroyed by phage action. It was soon noticed that, at least among the five most frequent mutant types as detected by phage resistance, there is a clear variation in tne morphology of the colonies. Although this variation might be continuous, the colonies can be classified into three fairly discrete groups: large, small, and tiny. Special experiments showed that the bacteria breed true as to which colony type they form. Taking into account colony type as well as resistance, we can say that about 20 distinct mutant types were present in our experiments. Evidence exists that *E. coli* is capable of producing a still greater variety of mutant types. BURNET and MCKIE (1936) worked with 37 different phage lines lysing one strain of *E. coli*, and there is very little doubt that this number could be increased. If there are mutants resistant to all types of phages, the number of different mutants that arise in *E. coli* must be considerable, probably running into hundreds.

As already stated, the different mutant types differ in frequency of occurrence. In the strain of *E. coli* that we used, the rate of mutation to **B**/2 is so low that we did not succeed in measuring it. The rates of mutation to **B**/(3, 4), **B**/(3, 4, 7), and **B**/6 range between 10^{-8} and 10^{-7}, while the rate of mutation to **B**/1 and **B**/(1, 5) is below 10^{-8}. This difference is significant. It is interesting to note that strains of bacteria which have by mutation become resistant to a certain phage still show rates of mutation to resistance to other phages similar to that of the original strain **B**. Thus, a line which had acquired resistance to

T6 (B/6) gives mutations leading to resistance to T_3, and thus is transformed into B/6/(3, 4) or B/6/(3, 4, 7), at a rate equal to that of the transformation of B into B/(3, 4) or B/(3, 4, 7). Furthermore, the multiple-resistant strains obtained in two or three steps from the original B line show a pattern of group resistance identical with that of the mutants obtained separately. It seems probable, therefore, that different mutants are independent of one another in origin and that they are caused by changes comparable to mutations in sexually reproducing organisms.

From the results obtained by us, it is evident that similar phenotypes may be obtained in different ways and may or may not have similar genotypes. For example, B/(1, 5) either may be obtained from B directly, by means of a single mutational step, or else may arise through summation of two independent changes: from B by mutation to B/1, and from B/1 by an independent mutation to B/1/5. Similarly, B/1, 3, 4, 5, 7 is occasionally obtained from B in one mutational step, but it can also arise in four steps: B to B/1, B/1 to B/1/5, B/1/5 to B/1/5/3, 4, and finally B/1/5/3, 4/7. On the whole, the observations suggest that two strains, B/1/5/3, 4/7 and B/1, 3, 4, 5, 7, although not distinguishable in their reactions to the phage lines at our disposal, actually possess different genotypes.

It may be questioned whether we are justified in referring to changes in the resistance of bacteria to the action of bacteriophages as "mutations," and particularly as "gene mutations." Since these changes are retained by the entire offspring of the modified colonies, and the properties of the changed strains are as stable as those of the original one, we are dealing with hereditary changes. Since these changes arise, as far as we can tell, in a single discrete step, it seems legitimate and proper to call them mutations. The justification for calling them gene mutations is much less convincing. The existence of genes in higher organisms is inferred from the Mendelian segregations in crosses between unlike strains or races; the unitary nature of a gene is defined by its behavior as a discrete unit in heredity, crossing over, mutation, and finally in chromosome breakage. Strictly speaking, it is the concurrence of all these criteria that permits us to retain the idea of discrete genes notwithstanding the evidence of their partial mutual dependence (position effects). Since bacterial reproduction, as far as is known, is exclusively asexual, inheritance in bacteria cannot be studied with the aid of the methods employed in work on higher organisms, nor can the criteria characterizing gene mutations be applied directly. We have given evidence, however, showing that several distinct types of mutational change arise in *E. coli*, each with its characteristic frequency and—what is perhaps most important in this connection—independently from one another. This is at least indicative of the existence in these bacteria of several independent self-reproducing and separately changeable entities, which to that extent remind one of genes as the latter are known in sexually reproducing organisms.

SUMMARY

In a single strain, B, of *Escherichia coli*, changes to resistance to seven strains

of bacteriophage (T1 to T7) were studied. A total of 377 mutants was investigated (table 1), and eight different groups were detected. Five of these—**B**/1, **B**/1, 5, **B**/3, 4, **B**/3, 4, 7, and **B**/6—occur with higher frequency than the other three—**B**/1, 3, 4, 5, **B**/6, 1, 5, and **B**/7, 1, 3, 4, 5. (The numbers following the bar indicate the phages to which the mutant is resistant.)

In addition, 364 mutants were derived from lines already resistant to one or more phages. The cross-resistance pattern in these tests does not significantly differ from the pattern obtained in the previous experiments, where mutants were derived from the sensitive **B** strain.

The rate of mutation to **B**/(1, 5) was approximately 10^{-8}, to **B**/(3, 4) between 10^{-8} and 10^{-7}, and to **B**/6 slightly higher.

Extensive tests made with 34 different mutants representative of the material used in this work indicate that the growth rates of mutants do not differ systematically from that of the original **B** strain, when they are grown together with it.

The rate of mutation to resistance to a given phage is similar for the **B** strain and for strains already resistant to some other phage or phages.

These results indicate that mutations to different resistance types are independent of one another and are probably produced by changes comparable to gene mutations.

Evidence indicates that complex mutants resistant to two or more phages may be obtained from **B**, either by means of a single mutational step or through summation of two or more changes.

It was found that mutants frequently show morphological differences in colony size, which breed true. At least three colony sizes can be distinguished for each of the eight types of mutants—namely, large, small, and tiny colonies. Altogether about 20 distinguishable mutant types showing some degree of resistance to our seven phages appeared in our experiments. Since many more phages affecting the **B** strain could be isolated, it is evident that the actual number of mutants affecting resistance is considerably larger.

A correlation between colony size and resistance type was noted. The frequency of small colony types is higher among **B**/(3, 4, 7) than among **B**/(3, 4) mutants.

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Before the experiments were completed, the junior author took leave of absence to join a war research project.

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THE RELATION OF REPEATS TO POSITION EFFECT IN DROSOPHILA MELANOGASTER

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INTRODUCTION

IN *DROSOPHILA MELANOGASTER* it has been established that the action of at least some genes is affected by their position in the chromosomes. Critical evidence for this phenomenon of position effect has been given by STURTEVANT (1925), DUBININ and SIDOROW (1935), and PANSHIN (1935). Position effects have been detected only in cases in which the relative position of a gene with respect to its neighbors has been altered by a chromosomal rearrangement. It is conceivable that the effective position of a gene may also be changed as a result of a substitution in an adjacent gene of one allele by another. Such a possibility was put to experimental test by STURTEVANT (1928) for the dominant mutants, Delta (*Dl*) and Hairless (*H*), three units apart in the third chromosome; however, no difference could be demonstrated between *Dl H*/++ and *Dl*+/+*H*.

On the other hand when a similar type of comparison is made with mutants at the Star and asteroid loci, a position effect can be detected, as will be shown below. The purpose of this paper is to present the evidence for an asteroid locus as distinct from that of Star, to examine the conditions which may make possible the detection of a position effect in this instance, and to study "changes" at the Star and asteroid loci associated with certain chromosomal rearrangements.

THE STAR AND ASTEROID MUTANTS

Star (*S*) is a spontaneous dominant mutant at 1.3 in the second chromosome (BRIDGES and MORGAN 1919; STERN and BRIDGES 1926). *S*/+ flies have slightly reduced eyes with irregularly shaped facets and disarranged facet hairs. The *S* homozygote is lethal. In a line of *S*/+ flies which had been inbred for several generations in mass cultures, several individuals appeared which had smaller eyes than those of *S*/+. An analysis of one of these showed that it carried *S* and, in the opposite chromosome, a dominant intensifier of *S*. By itself this factor behaved as a recessive, rough-eyed mutant in the left end of the second chromosome. This mutant, formerly called Star-recessive (S^r), is known from evidence presented below to be close to the right of *S* and has been renamed asteroid (*ast*).

The compound *ast*/+ occasionally has a very slightly roughened eye, but it is usually inseparable from wild type. The eyes of flies homozygous for *ast* are smaller than those of *S*/+ and the longitudinal wing veins, L_2, L_3, L_4, and L_5, occasionally have terminal gaps. The compound, *S*+/+ *ast*, has an extremely small, diamond-shaped eye with many of the facets fused and the rest

of irregular size and distribution. The longitudinal veins, which are sometimes interrupted in *ast/ast*, usually have large terminal gaps in *S+/+ ast*.

Asteroid^2 (*ast^2*) is probably an allele of *ast*, although the possibility that it is a recessive allele of *S* has not been excluded. It appeared spontaneously in a single F_1 male from a mating of *ast* females to *al S ho/Cy* males (*al*, aristaless at 0.0; *ho*, heldout at 4.0). The fly had Curly (*Cy*) wings and rough eyes and was therefore readily distinguishable from the *Cy/ast* class. From an identical type of mating, conducted at a later time, another allele of *ast*, *ast^3*, arose in the first or second generation and was detected in the same way as was *ast^2*. In the case of both *ast^2* and *ast^3* it was shown that each had arisen in a Curly inversion chromosome. Although these mutants have not been separated from the inversion in the left arm of the second chromosome, they have been freed of the Curly mutant. Homozygous *ast^2* and homozygous *ast^3* types have good viability but the former is sterile in the female; in each case the eye effect is similar to that of homoyzgous *ast*, but the wing venation is normal. The compound, *S/ast^2* is lethal. The *S/ast^3* types are late-hatching, but those which emerge have good viability and fertility; they have normal venation and extremely small eyes which are similar to, but slightly larger than, those of *S/ast*.

In a search for spontaneous reverse mutations of *ast* to *ast$^+$* from homozygous *ast* females, a slight allele of *ast*, *ast^4*, appeared in a single F_1 male of composition, *al S ho/ast^4 ho*, from a cross of *al ast ho/ast* females to *al S ho/Cy* males. Here the calculated number of offspring in which *ast^4* could be detected was approximately 15,700, and the observed recombination value for the *al–ho* interval was 5.5 percent. The possibility that the observed association of mutation with crossing over in the *al-ho* region was due to chance is perhaps strengthened by the fact that an allele, *ast^5*, which closely resembled *ast^4*, arose in a noncrossover individual of composition, *al ast ho/al ast^5 ho*, from a mating (conducted at 30°C) of *al ast ho/ast* females to *al ast ho* males. *Ast^4/+* and *ast^4/ast^4* are wild type, although the latter may become as extreme as *ast/ast* under unfavorable culture conditions. The compound, *S/ast^4*, has a smaller eye than that of *S/+* and resembles rather closely *ast/ast* not only in having a similar eye effect but also in occasionally producing a similar pattern of gaps at the tips of the longitudinal veins.

The phenotypic effects of all possible combinations of *+*, *S*, *ast*, *ast^2*, *ast^3*, and *ast^4* are indicated in table 1 by means of a rough, arbitrary grading of the eye effect, based on inspection rather than measurement. Wild type is taken as Grade 1. Grade 2 has a very slight facet irregularity. Higher numbers indicate decreasing size and increasing roughness of the eye. The possibility that modifiers at other loci are a complicating factor in the phenotypic expressions of the various *ast* alleles has been excluded rather rigorously in the case of *ast*, and to some extent for *ast^2*, *ast^3*, and *ast^4*.

In summary, the alleles, *ast* and *ast,^{4}* are more nearly similar to each other than to *ast^2* and *ast^3* in having good viability when opposite *S*, a variable expression, and a tendency to interrupt the wing venation; whereas, *ast^2* and *ast^3* have peculiar lethal effects with *S* and, by themselves, a relatively constant expression and no perceptible effect on the wing venation.

TABLE 1

A rough classification of eye types of various combinations of S and ast alleles, including those with Enhancer of Star, E-S. The extent of variability is indicated, where necessary, in parenthesis. Asterisk (*) indicates that individuals of type in question may show gaps in the longitudinal wing veins.

	S^+ ast^+	S^+ ast^1	S^+ ast^3	S^+ ast	S^+ ast^2	S ast^+	S^+ ast^+ E-S
S^+ ast^+	1						
S^+ ast^1	1	1 (1–5)*					
S^+ ast^3	1	2 (2–3)	5				
S^+ ast	1 (1–2)	3 (1–5)*	4 (4–6)	5* (1–7)			
S^+ ast^2	1	4 (3–5)	5	6 (4–8)*	5 (♀, sterile)		
S ast^+	3 (2–3)	5* (4–6)*	7 (late hatching)	8 (6–8)*	lethal	lethal	
S^+ ast^+ E-S	1	2	2	4	3	5 (5–6)	2

In table 1 the interactions of *S* and the *ast* alleles with the dominant, Enhancer of Star (*E-S*, at 6 ± in the second chromosome) are also shown. The relative specificity of *E-S* in strongly enhancing *ast*/+ and *ast*^{2}/+ is shown by the fact that it had, when heterozygous, little, if any, such effect on other recessive rough-eyed mutants which were tested in the heterozygous condition. The mutants thus tested were: echinus, facet, split, rough^{ex}, uneven, morula, roughish, rolled, rubroad, scabrous, rough, and roughoid. Another interaction, not shown in table 1, is that with either homozygous net or homozygous plexus, each of which is partially suppressed by *S*/+ and tends to be completely suppressed by *ast*/*ast* and *S*/*ast*.

BRIDGES (1936) has reported that *S* is apparently normal in the salivary gland chromosomes. Since the *S* and *ast* loci are now known to lie in the region of the 21E1–2 doubtlet, particular attention has been paid to this region in a cytological study of *S*, *S*^{2} and *S*^{r} (two alleles closely resembling *S*^{1}), *ast*, *ast*^{2}, *ast*^{3}, and *ast*^{4}. Each of these mutants has been found to be apparently normal in the salivary gland chromosomes.

THE EVIDENCE FOR AN ASTEROID LOCUS

Data summarized in table 2 represent part of the evidence that *ast* and *ast*^{4} are located close to the right of *S*, and they suggest that *ast*^{3} is located close to the right of *S*^{2}. The first indication that *ast* was not an allele of *S* was the appearance of a single+/*ast* fly among 3,235 offspring of a cross of *S*/*ast* females to *ast* males (Mating 1). Additional data were then collected from *al S ho*/*ast* females which were individually mated in separate experiments to *ast*, *al ast ho*, and *al S ho*/*Cy* males with the results shown in Matings 2a, 2b, and 2c, respectively. Mating 2a produced a normal fly which was shown by a cross to *al S ho*/*Cy* to have had the probable composition, *ast*/*ho*; seven other apparently normal offspring from Mating 2a were shown by the same test to have been instances in which homozygous *ast* had overlapped wild type. Mating 2b yielded no normal-eyed offspring. From Mating 2c, two non-*Cy* females with *ho* wings and eyes resembling those of *S*/+ were recovered in separate cultures. These females, which were non-virgin, were separately mated to *al S ho*/*Cy* males; in each case, some of the F_1 flies were phenotypically identical with the parental female. In one case a pair mating of these F_1 flies was made, and it produced an F_2 which had "*S*/+" eyes and normal eyes in the ratio, 2:1. Thus the females produced in Mating 2c had the probable composition, *al S ho*/*ho*.

In Mating 2 the "+" types, which may be interpreted as wild type crossovers between *S* and *ast*, occurred with a frequency of 0.009 per cent. Here the total number of offspring in which the "+" types could be detected is calculated, as shown in Column 3, from that class which had approximately the same viability as those types—namely, the "*ast*" offspring in Matings 2a and 2b, and the "*Cy*" class in 2 c.

A more satisfactory method of recovering the "+" crossovers was employed in Mating 3, table 2. Here the parental *S*/*ast* females were heterozygous for the mutants, *net* (net, at 0.0–), *dp* (dumpy, at 13.0) and *cl* (clot, at 16.5), as well as for *al* and *ho*. These mutants were arranged to give the approximately alternated composition, *net S dp cl*/*al ast ho*. In addition, the parental females were made heterozygous for inversions in chromosome arms other than 2*L*, since this procedure was known to increase the frequency of crossing over between *al* and *ho*. In order to obtain females of the required composition, *al ast ho In*(2*R*)*Cy*/*Cy*, *E–S* females were mated to *In*(1) *dl-49*, *cm*^{2}/*Y*; *net S dp cl*/*Cy*(2*L*) *dp*^{2} *b pr*; *In* (3*L*+3*R*)*P*/+ males. The F_1 *In* (1) *dl-49*, *cm*^{2}/+; *net S dp cl*/*al ast ho In* (2*R*) *Cy* females, of which one-half are expected to be heterozygous for the Payne inversions in both arms of chromosome 3, constituted the parental females employed in Mating 3. The parental males in this mating were homozygous for *In* (2*L*) *Cy*, *ast*^{2}. Since *S*/*ast*^{2} is lethal, the use of *ast*^{2} rather than *ast* served to reduce by one-half the total number of offspring to be examined for the "+" crossovers. At the same time, *ast*^{2} made easier the detection of such crossovers, since *ast*^{2}/*ast*, in contrast to *ast*/*ast*, had not been observed to overlap wild type.

In addition to 16,068 "*ast*/*ast*^{2}" offspring, Mating 3 yielded 15 flies with normal eyes. Of the latter, one was sterile and another (with Minute bristles)

was lost. The remaining 13, composed of eight males and five females, were individually outcrossed to an *al S ho/Cy, E-S* stock. In each case, the F_1 non-*Cy* flies, except for those expected on the basis that the tested individual was a non-virgin female, were aristaless and had the "*S*/+" type of eye. Two types of *Cy* flies, those with normal eyes and those of the expected "ast²/*E-S*" type were obtained in the F_1 of each of these matings. An F_2 was raised from each

TABLE 2

The frequency of "+" crossovers between the S and ast loci. The inversions for which the parental females were homozyous are shown in column 2. Calculated total progenies (column 3) are based on total counts of the class shown in parenthesis. The number and composition of the tested crossover chromosomes are indicated in parenthesis in column 4.

	MATING	INVERSIONS	TOTAL	WILD TYPE CROSSOVERS	PERCENTAGE
1	$\frac{S\ +}{+\ ast}$ ♀ × *ast* ♂♂	Unknown	2×1,728 ("*ast*")	1(?)	
2a	$\frac{al\ S\ +\ ho}{+\ +\ ast\ +}$ ♀ ± *ast* ♂♂	Unknown	2×6,933 ("*ast*")	1 (*ho*)	
b	(") ♀ × *al ast ho* ♂♂	Unknown	2×2,038 ("*ast*")	0	0.009
c	(") × $\frac{al\ S\ ho}{Cy}$ ♂♂	Unknown	15,753 (*Cy*)	2 (2, *ho*)	
3	$\frac{net\ +\ S\ +\ +\ dp\ cl}{+\ al\ +\ ast\ ho\ +\ +}$ ♀ × *In*(2L)*Cy, ast²* ♂♂	*In*(1)*dl-49, cm²*; *In*(2R)*Cy, cm²*; and in about ½ ♀♀, *In*(3L+3R)*P*.	2×16,068 (*ast*/*ast²*)	15 (12, *al dp cl*)	0.047
4	$\frac{net\ S\ +\ +\ dp\ cl}{+\ +\ ast^4\ ho\ +\ +}$ ♀ × *In*(2L)*Cy, ast²* ♂♂	*In*(1)*dl-49*, cm²; and in some, *In*(3L+3R)*P, ru*.	2×7,656 (*ast⁴*/*ast⁴*)	2 (2, *dp cl*)	0.01
5	$\frac{+\ S^3\ +\ In(2L)t}{al^2\ +\ ast^3\ In(2L)Cy}$ ♀ × $\frac{al\ S\ ho}{Cy, E-S}$ ♂♂	*In*(1)*dl-49, cm²*; *In*(2R)*Cy, cm²*; and in about ½ ♀♀, *In* (3LR)*sep, ri p²*.	17,334 (*Cy*)	7 (4, *al² In−1*)	0.04

of 12 of the 13 progeny tests by mating the *Cy*, normal-eyed flies *inter se*. The F_2 non-*Cy* offspring had in each case normal eyes and were phenotypically *al*, *dp* and *cl*. Thus, at least 13 "+" crossovers between *S* and *ast* were recovered in Mating 3, of which 12 were shown to carry the marker *dp* and *cl*, as well as *al*. It will be noted that the composition of the crossover types is expected to remain intact in the above progeny tests since those types were constantly kept against the Curly inversion in the left arm of chromosome 2. Stocks of two separate occurrences of the "+" crossover types from Mating 3 were kept for further tests. These included matings to *ast*, *ast³*, and *ast⁴* and to the *S* deficien-

cies of *Df-S1* and *Df-S2*, which will be described later. The results of each of these matings were consistent with the assumption that the "+" crossovers carried the normal alleles of *S* and *ast*. A cytological analysis of these two stocks showed no departure from normal in the critical *S* region.

The results of Mating 3 are consistent with those of Mating 2 in indicating that *ast* is close to the right of *S*. The frequency of normal-eyed flies in Mating 3 was 0.08 per cent. Since the S/ast^2 class is lethal, the calculated frequency of "+" crossover types is 0.04 per cent. Inspection of table 5 shows that significant increases in crossing over in the *al–ho* region are obtained by the method adopted in Mating 3 of introducing inversions into other chromosome arms. The increase in frequency of "+" crossovers in Mating 3, compared to Mating 2 can be best attributed to this procedure.

It was further found that $S+/+$ ast^4 females produced occasional "+" crossover types (Mating 4). The original ast^4 *ho* strain, kept by mass inbreeding was used as the source of ast^4. The "+" types were detected and tested by methods analogous to those used in Mating 3, although a less "efficient" inversion setup was employed. Only two normal-eyed flies were recovered in this experiment. Each was found to be a "+" crossover type similar to those obtained from $S+/+ast$ females. The lower frequency—namely, 0.01 per cent—of "+" crossovers from $S+/+ast^4$ females as compared with 0.04 per cent from $S+/+$ *ast* females is at least consistent with the use of fewer inversions in Mating 4 as compared to Mating 3. In their interactions with *ast*, ast^2, ast^3, *S* and *E–S*, the "+" crossovers from Mating 4 behaved as though they carried the normal alleles of *S* and *ast*. Likewise their cytological picture was apparently normal for the *S* region of the salivary gland chromosome.

Technical difficulties arising from the association of S^2 and asteroid^3 with *In(2L)t* and *In(2L)Cy*, respectively, prevented a study of their crossing over relationships with respect to either *ast*, ast^4, or *S*. However, since the sequences in these two inversions are nearly identical to each other (BRIDGES and LI 1936), the linkage relations of S^2 and ast^3 could be determined directly. For this purpose, *In(2L)Cy*, al^2 ast^3 *b pr* (homozygous) females were crossed to *In(1)dl-49*, cm^2/Y; *In(2L)t*, S^2*In(2R) Cy/Cy(2L)*dp^2 *b pr*; *In(3LR)sep*, *ri* p^p *sep* males. The F_1 "S^2/ast^3" females, one-half of which are expected to carry the third chromosome "separated" inversion of MULLER were individually mated to *al S ho/Cy*, *E-S* males with the results shown in Mating 5. Since S^2/S is lethal, only three general types of offspring were recovered. In addition, a total of seven non-Curly, aristaless flies resembling "$S/+$" were detected in the F_1.

It will be noted by reference to figure 1 that the left break points of *In(2L)t* and *In(2L)Cy* each lie in the region to the left of *ho* and to the right of *S*. Therefore, although no marker mutants close to the right of *ast* are employed in the parental females, that region is strategically marked by the inversions themselves. Of the seven "+" types, four were successfully studied cytologically. The procedure here was separately to cross the aristaless "$S/+$" flies to an *al S ho/Cy*, *E-S* stock. The F_1 Curly males were backcrossed to *al S ho/Cy*,

E-S females. In each of the four tests some of the F_2 larvae carried the overlapping inversion complex, *In(2L)t/In(2L)Cy*. At the same time it was determined that each of the "+" crossover types was apparently normal in the critical 21E region. The "+" crossovers were mated to *ast* and *ast*^{3} (as well as to *S* and *E-S*), and their behavior was that expected on the basis that they carried the normal alleles of *S* and *ast*. Thus the probable composition of each of the four crossover chromosomes was *al*^{2} *S*$^+$ *ast*$^+$ *In(2L)t*. The frequency of the "+" crossovers can best be computed on the basis of the Curly offspring which

TABLE 3

The frequency of "S ast" and "S ast^4" crossovers. (See text and table 2 for description.)

	MATING	INVERSIONS	TOTAL PROGENY	"S as" OR "S ast^4" CROSSOVERS	PER-CENTAGE
6	$\frac{al\ S\ +\ ho}{+\ +\ ast\ +}$ ♀ × *al ho* ♂♂	Unknown	2,967	0	—
7a	$\frac{net\ +\ S\ +\ +\ dp\ cl}{+\ al\ +\ ast\ ho\ +\ +}$ ♀ × *al ho* ♂♂	*In* (1) *dl-49, cm*^{2}; *In* (2R)*Cy, cn*^{2}; *In(3L)P, Me* *In* (3R)*C, Sb e*	3,005	0	—
b	(") ♀ × *al Dp−S ho* ♂♂		6,222	0	—
8	$\frac{net\ S\ +\ +\ dp\ cl}{+\ +\ ast^4\ ho\ +\ +}$ ♀ × *al ho* ♂♂	*In* (1)*dl-49, cm*^{2}; and in some, *In* (3L+3R)*P, ra*	2,914	0	—
9	$\frac{+\ al\ (S\ ast)\ (+\ ast)\ ho\ +\ +}{net\ +\ +\ ast\ +\ +\ dp\ cl}$ ♀ × *al go* ♂♂	*In*(1)*dl-49, cm*^{2}; *T*(2; 3)*Me, Me*	4,864	3 (2, *al S ast dp cl*)	0.06
		In(1)*dl-49, cm*^{2}; *T*(2; 3)*Me, Me*/ *In* (3LR)*CxD, D*	1,531	2 (1, *al S ast dp cl*)	0.1
10	$\frac{al\ (S\ ast)\ (+\ ast)\ ho\ +\ +}{+\ +\ ast^4\ +\ dp\ cl}$ ♀ × *net* ♂♂; *net ho* ♂♂; and *al ho* ♂♂	*In*(1)*dl-49, cm*^{2}; *T*(2; 3)*Me, Me*/ *In* (3LR)*sep, p*^{p}.	6,667	3 (3, *al S ast*^{4} *dp cl*)	0.04

totaled 17,334. This gives an estimated frequency of 0.04 per cent (7/17,334). These results, when compared with those of Matings 2 and 3, indicate that *S*^{2} is probably an allele of *S* and not a dominant allele of *ast*; similarly, *ast*^{3} probably is an allele of *ast* rather than a recessive allele of *S*.

At first attempts to recover crossovers having *S* and *ast* or *S* and *ast*^{4} in the same chromosome failed. These experiments, shown in table 3, Matings 6, 7, and 8, were conducted on the assumption that *S ast*/++ or *S ast*^{4}/++ would resemble phenotypically *S*+/+ *ast* or *S*+/+*ast*^{4}, respectively, or that they would be at least more extreme than *S*+/++. In Mating 7b, use was made of a suppressor of *S*, *Dp-S*, in the parental males, on the basis that it might permit the survival and detection of *S ast*. However, no offspring with eyes more extreme than "*S*/+" (or wild type in the case of Mating 7b) were detected in any of these experiments among a total of 15,108 offspring.

An indirect way of obtaining the *S ast* and *S ast*^{4} combinations suggested itself as a result of independent studies of the tandem Star Duplication, *Dp-S* (LEWIS 1941). To understand the method employed, some of the properties of this duplication require review.

A combination of genetic and cytological studies now indicates that this duplication includes two known loci, *S* and *ast*. Cytologically, this aberration is a tandem repeat in direct order for the four bands: 21D3,4 and the 21E1–2 doublet structure or "capsule" (see fig. 1). As originally obtained, this duplication, which arose from an *ast* female, had the probable composition, (+*ast*) (+*ast*), formerly written (S^r)(S^r). This notation is to be taken as indicating that the regions in parenthesis are in adjacent, direct order and that each carries the normal allele of *S* and the recessive *ast*. Many variations in the genetic composition of *Dp-S* have been obtained, but for present purposes only the derivative, (*S ast*)(+*ast*), formerly written (*S*)(S^r), need be considered. This derivative was obtained among the offspring of a mating in which (+*ast*)(+*ast*) *ho/net S dp cl* females were individually mated to *In(2L)Cy*, *ast*^{2} *b pr* males. The great majority of the F_1 had normal eyes—that is, the (+*ast*)(+*ast*)/*ast*^{2} class; in addition, there were 206 offspring with eyes resembling "*ast/ast*^{2}." Four possibilities seemed likely for the origin of these types. Thus, by unequal crossing over, *ast* could have been extracted from the duplication in two ways, either as (1) *ast dp cl*, or as (2) *net ast ho*. *S* could likewise be inserted into the left section of the duplication in two ways, either as (3) *net* (*S ast*)(+*ast*) *ho*, or as (4) *net* (*S*+)(+*ast*) *ho*. Two other duplication types possible here—namely, (+*ast*)(++) and (+*ast*)(*S*+)—were known to be wild type opposite *ast*^{2} and therefore could not be detected in this experiment. Of the 206 cases, 192 were successfully tested to a *net ast ho* stock. In each of these progeny tests some of the F_1 flies were phenotypically *net ast ho* (except for two which were *ast ho*). Thus the first possibility, the extraction of *ast* from the left section of the duplication, was not realized, A salivary gland chromosome analysis was then made of 83 of the "*ast*" crossover types. Only two of these proved to carry *Dp-S*. These were indistinguishable phenotypically from the 81 *net ast ho* types which did not carry the duplication. Since type (4) is complementary to type (1), which did not occur in this or related experiments, it can be assumed that the composition of these two duplication types was (*S ast*)(+*ast*). This genetic structure, at least with respect to the *S* locus, was confirmed by extracting from the left (distal) section of the repeat the newly introduced *S*. This was done in three ways. The results of two of these methods are shown in Matings 9 and 10 of table 3. Females of composition, *al* (*S ast*)(+*ast*) *ho/net ast dp cl* (Mating 9) or *al* (*S ast*)(+*ast*) *ho/ast*^{4} *dp cl* (Mating 10) when mated to *al ho* males produced for the most part only normal-eyed flies. However, each type of female produced several aristaless offspring with eyes resembling those of "*S*/+" flies. These rare types were also produced when *al*(*S ast*)(+*ast*)/*dp cl* females were mated to *al ho* males. No types with eyes more extreme than "*S*/+" were detected in any of these experiments.

In the next step, three separate occurrences of what may be called the "*S*" crossovers from Mating 9, and two such cases from Mating 10 were tested for the presence of *ast* or *ast*^{4}. The results of these five tests are grouped and summarized in Matings 11, 12, 13, and 14 of table 4. Matings 11 and 12 were tests of "*S*" crossovers derived from Mating 9 and in each of these three tests one (*net*) *ast dp cl* crossover was recovered. Likewise, at least one *net ast*^{4} *dp cl* crossover was detected from each of the two tests of "*S*" crossovers from Mating 10.

These linkage studies indicated that the probable composition of the "*S*"

TABLE 4

The frequency with which ast and ast^4 *were recovered from S ast /++ and S ast*^{4}*/++ females, respectively. (See text and table 2 for description.)*

	MATING	INVERSIONS	TOTAL	"*ast*" OR "*ast*^{4}" CROSSOVERS	PERCENTAGE
11	$\frac{al\ \ S\ ast\ \ dp\ \ cl}{+\ +\ +\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*T(2;3)Me, Me*	2×2,304	1 (*ast dp cl*)	0.02
12	$\frac{+\ \ al\ \ \ S\ ast\ \ +\ \ dp\ \ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ × *al ast ho* ♂♂ and *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In(1)dl-49, cm*^{3}; *Dp(2)T(2;3)Me, Me*	2×1,594	2 (2, *net ast dp cl*)	0.06
13	$\frac{+\ \ al\ \ \ S\ \ ast^4\ +\ \ dp\ \ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In (1) dl-49, cm*^{3}; *Dp(2)T(2;3)-Me, Me*	2×2,754	4 (3, *net ast*^{4} *dp cl*; 1, *net ast*^{4})	0.07
14	$\frac{net\ +\ \ S\ ast\ \ \ \ \ \ \ +\ dp\ \ cl}{+\ al\ \ (+\ ast)(+ast)\ ho\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In(1)dl-49, cm*^{3}/ *In(1)AM, ptg*^{4}; *T(2;3)Me, Me*	2×996	1 (*al ast dp cl*)	0.05

crossovers from Matings 9 and 10 were *al S ast dp cl* and *al S ast*^{4} *dp cl*, respectively. In these studies the *ast* and *ast*^{4} crossovers were derived from *S ast/++* and *S ast*^{4}*/++* females, respectively. Still another *S ast* crossover type from Mating 9 was tested in Mating 14 by a different procedure, but one analogous to that by which the *S ast* type was first recovered. In this case *net S ast dp cl/al* (+*ast*)(+*ast*) *ho* females were mated to *In(2L)Cy, al*^{2} *ast*^{3} *b pr* males. Here, too, it was possible to recover an individual whose composition was shown to be *al ast dp cl/In(2L)Cy, al*^{2} *ast*^{3} *b pr*. The evidence that the rare crossover types obtained in the matings shown in table 3 carried *ast* or *ast*^{4} was based on phenotypic studies which included obtaining homozygotes for the crossover chromosome as well as testing to *S+*, *S ast*, and *S ast*^{4}.

A cytological study was made of five of the *S ast* types from Mating 9, two *S ast*^{4} types from Mating 10, and several of the *ast* and *ast*^{4} crossovers from Matings 11–13. None of these carried *Dp-S*, and, with the exception of one *S ast* type, all were apparently normal in the critical *S* region of the salivary gland chromosomes. The exception here showed a slight disturbance

near the 21E1–2 doublet. The exact nature of this irregularity was not determined but it appeared to involve a duplication or inversion of a single faint band. This aberrant *S ast* type was not included in the crossover studies shown in table 4; however, a cytologically normal *ast* crossover was extracted from it by the same procedure as that used in the other cases.

In their phenotypic reactions, *S ast* and *S ast*^{4} were indistinguishable from

TABLE 5

A comparison of the standard recombination values (together with their standard deviations) for the al-ho region, with those obtained when the parental females were heterozygous for various inversion (or translocation) complexes.

NUMBER OF MATING	SEQUENCE IN MAJOR CHROMOSOME ARMS OF PARENTAL ♀♀					PERCENTAGE RECOMBINATION	
	X	2L	2R	3L	3R	*al-S*	*al-ho*
6	(standard)					1.2±0.2 (36/2,967)	4.7±0.4 (140/2,967)
7a, b	*dl-49* / +	+ / +	*Cy* / +	*P* / +	*C* / +	4.3±0.4 (128/3,005)	10.9±0.3 (1,011/9,227)
12, 13	*dl-49* / +	+ / +	+ / +	*Dp(2)T(2;3)Me* / +		4.2±0.3 (167/4,008)	12.1±2.7 (18/149)
—	+ / +	*Dp-S* / +	+ / +	+ / +	+ / +	—	4.3±0.2 (343/8,000)
9	*dl-49* / +	*Dp-S* / +	*T(2;3)Me* / +; +			—	13.8±0.6 (455/3,297)
9	*dl-49* / +	*Dp-S* / +	*T(2;3)Me* / +; *In(3LR)CxD*			—	17.3±1.7 (85/490)

S ast$^+$ in tests to +, *E-S*, *ast*^{2} and *ast*^{3}. All combinations in which *S* was homozygous were lethal regardless of the *ast* composition. However, opposite *ast*, and similarly opposite *ast*^{4}, *S ast*^{4} produced a larger eye than *S ast* which in turn produced a larger eye than *S ast*$^+$. Thus, the following sequence, arranged in order of decreasing eye size, could be established: *S ast*^{4}/+*ast*^{4}, *Sa st*/+*ast*^{4}, *S*+/+*ast*^{4}, *S ast*^{4}/+*ast*, *S ast*/+*ast*, and *S*+/+*ast*. The largest eye produced here is still somewhat smaller than that typical of *S*/+.

It was felt that the difference in eye size between *S ast*/+*ast* and *S*+/+*ast* (or between *S ast*/+*ast*^{4} and *S*+/+*ast*^{4}) was not sufficiently distinct owing to

the inherent variability of these types to make practicable the detection of an *S ast* crossover from a mating of *S+/+ast* females to *ast* (or *ast*^{4}) males. However, it was possible to recover *S ast*^{4} from *S+/+ast*^{4} females on the basis of the more distinct difference existing between *S ast*^{4}*/+ast* and *S+/+ast*. In this experiment, mating 4a, not shown in the tables, *In(1)dl-49, cm*^{2}*/+; al S ho/ast*^{4} *dp cl; In(3LR)sep/+* females were mated to *al ast ho* males. Total counts were not made but among a large number of offspring several aristaless flies were obtained which had eyes intermediate between *S+/+ast* and *ast /ast*^{4}. Three of these upon further testing were found to carry the desired *S ast*^{4} crossover, on the basis that it behaved phenotypically exactly like the *S ast*^{4} types obtained from Mating 10. The remaining cases were found to be instances in which *S/ast* had overlapped wild type.

In addition, a stock of one of the complementary *ho* crossovers which occurred in Mating 15 was established. It was then possible to make up flies having the composition *al S ast*^{4} *dp cl/ho* whose second chromosomes were the two complementary crossovers derived from mating 4a. The phenotype in this case was again indistinguishable from *S ast*$^+$*/++*.

In summary, some 39 crossovers were obtained between the Star and asteroid loci. Twenty-two of these were "+" crossovers derived from *S+/+ast*, *S+/+ast*^{4}, or *S*^{2}*+/+ast*^{3} females. Their frequency varied between 0.009 per cent in the absence of inversions in the parental females to 0.047 per cent when the parental females were heterozygous for *In(1)dl-49*, *In(2R)Cy*, and, in some, *In(3L+3R)P*. Six *S ast* or *S ast*^{4} crossovers were detected in the progeny of *(S ast)(+ast)/ast* and *(S ast)(+ast)/ast*^{4} females, respectively. The frequency varied between 0.04 and 0.1 per cent, depending on the particular inversions present.

A SURVEY OF THE EXTREME LEFT END OF CHROMOSOME 2

Early in the genetic analysis of *S* and *ast*, an attempt was made to correlate those loci with the salivary gland chromosome structure in the left end of chromosome 2. This correlation was particularly necessary as a basis for a cytological analysis of the *S* and *ast* mutants. Genetic and cytological correspondence were obtained not only for *S* and *ast* but also for most of the other mutant loci in their immediate vicinity. A genetic map of this region, which includes approximately the first four map units of the left end of chromosome 2, is shown as the top line in figure 1. This section includes at least ten known loci. Descriptions and linkage data for the mutants, aristaless (*al*), expanded (*ex*), and dachsous (*ds*), as well as for Star, have been given by STERN and BRIDGES (1926). The mutant, telegraph (*tg*, at 0.0±), also described by these authors, has been lost and is omitted from the linkage map shown here. An account of net (*net*), shrunken (*shr*), heldout (*ho*), and lethal giant larvae (*l-gl*), together with BRIDGES' revised location of *l-gl* at 0.0±, is given by BRIDGES and BREHME (1944).

The drawing, in figure 1, of the first two sections of chromosome 2L is

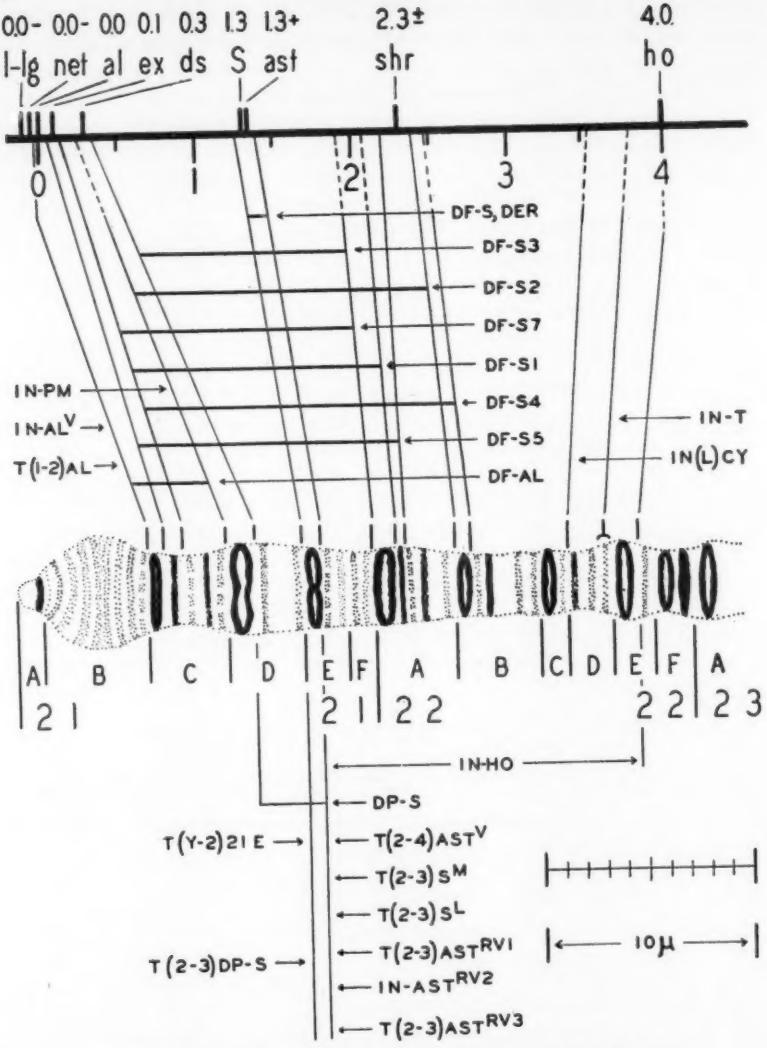

FIGURE 1.—A correlation of the linkage map of the extreme left end of the second chromosome with the salivary gland chromosome structure.

semi-diagrammatic and composite, based on moderately stretched chromosomes which were stained with aceto-orcein according to the method of LA COUR. The chromosome is labeled to correspond with BRIDGES' (1935) map.

Reference to the latter map shows that the section extending from 21A to just after 23A1–2 corresponds, as calculated by BRIDGES, to about 4.4 map units on the genetic map. In figure 1, this value has been used as the basis for the correlation of the genetic and cytological maps.

Much of the material which has made possible the genetic and cytological correspondences shown in figure 1 was obtained from an experiment in which adult wild type (Canton-S) males were X-rayed (3,000 r units) and mated to *al ast ho* females. The results of this experiment are summarized in table 6. Total counts were not made; however, a random sample of 16 out of 196 cultures, in which the parental males were allowed to remain for the first five days, averaged 82 flies per culture. A total of 103 transfer cultures produced an additional 3,398 flies. Therefore the combined total number of offspring

TABLE 6

Results from X-rayed (3,000 r units) wild-type ♂ × al ast ho ♀. Total offspring: approximately 19,000. (Df = Deficiency, In = Inversion, T = Translocation.)

CHANGES RESEMBLING:	NUMBER NOT ANALYZED	NUMBER ANALYZED				TOTAL
		ASSOCIATED WITH ABERRATIONS			"POINT MUTATIONS"	
		Df	*In*	*T*		
aristaless	5	1	1	0	0	7
Star	5	5	0	1	1?	12
asteroid	4	0	0	1	1?	6
heldout	9	0	1	0	0	10
Notch	13	—	—	—	—	13

was roughly 19,500. F_1 aberrant individuals, in which the eyes were smaller and rougher than in *S*/+ flies, or whose phenotypes resembled *al* or *ho*, were separately crossed to an *al S ho*/*Cy*, *E-S* stock. A preliminary examination was made of the salivary gland chromosomes of F_1 larvae from each of the fertile progeny tests. This was followed up in most cases by a more detailed cytological analysis after a balanced culture had been established.

No substantial case of "point mutation" to either *al* or *ho* was detected in this experiment. A possible change of this type to a *S* and to an *ast* allele, simultaneously, will be discussed later. The following is a description of those analyzed changes shown in table 6 which were found to be accompanied by a chromosomal aberration; an additional *S* deficiency (*Df-S7*) obtained from a related X-radiation experiment is also included:

Df-al-Deficiency (*2*) *aristaless*.—LEWIS 1940. *Df*/+ is an extreme Minute with rough eyes and slight *ex*-and *ds*-like effects. Deficient for *al*, *ex* and *ds;* but not for *l-gl*, *net* or *S*. *Df-al*/*net* has a weak net effect. The loss extends from just before the 21C1–2 doublet to just before the 21D1–2 doublet.

Df-S1-Deficiency (*2*) *Star.*—LEWIS 1940, *Df*/+ has a slightly smaller and rougher eye than "+" and is less extreme and more variable than "*S*/+"; viability and fertility are good. *Df/ast* is somewhat less extreme than *S*+ /+*ast*, and more like *S ast*/+*ast;* otherwise the *Df* acts exactly like *S*. Deficient for *ds*, *S*, and *ast;* but not for *l-gl*, *net*, *al*, *ex*, *shr*, or *ho*. The breaks follow the medium 21C3 band and just precede the heavy 22A3 band.

Df-S2-Deficiency (*2*) *Star-2.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *ds*, *S*, *ast*, and *shr;* but not for *l-gl*, *net*, *al*, *ex*, or *ho*. The loss appears to extend from just to the left of 21D1–2 doublet to just preceding the 22B1–2 doublet. It is also possible that the breaks occur in the middle of these two doublets.

Df-S3-Deficiency (*2*) *Star-3.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *S* and *ast;* but not for *l-gl*, *net*, *al*, *ex*, *ds*, *shr*, or *ho*. The loss extends from just to the right of the 21D1–2 doublet to just before the 22A1–2 doublet.

Df-S4-Deficiency (*2*) *Star-4.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *ds*, *S*, *ast*, and *shr;* but not for *l-gl*, *net*, *al*, *ex*, or *ho*. The breaks follow the medium 21C3 band and the 22B1–2 doublet.

Df-S5-Deficiency (*2*) *Star-5.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1* except that *Df-S5*/+ has a slight *ex*-like effect. Deficient for *ex*, *ds*, *S* and *ast;* but not for *l-gl*, *net*, *al*, *shr*, or *ho*. The breaks occur just after the 21C1–2 doublet and after the heavy 22A3 band.

In(2LR)al^{V}—Inversion (*2LR*) *aristaless-variegated.*—LEWIS 1940. *al^{V}/al* is similar to homozygous *al;* however, in the presence of an extra *Y* chromosome, *al^{V}/al* is wild type. The homozygote is lethal. *Df-al/al^{V}* is viable and variegated in that sometimes only one instead of both of the aristae is missing. The euchromatic break appears just to precede the 21C1–2 doublet; the right break is in the heterochromatin of chromosome 2R.

T(2;3)S^{L}—Translocation (*2;3*) *Star.*—LEWIS 1940. *S^{L}*/+ resembles *S*/+; *S^{L}* also resembles *S ast^{+}* opposite *ast*, *ast^{2}*, *ast^{3}*, *ast^{4}*, and *E-S*. This is a complex rearrangement involving at least three breaks: one follows the 21E1–2 doublet of chromosome 2L; chromosome 3R has a break which apparently just precedes 88D8 and another in the basal heterochromatin. The tip of chromosome 2L up to and including 21E1–2 is translocated to heterochromatin of chromosome 3, but it has not been possible to determine from a salivary gland chromosome analysis whether the section from the base of 3R to 88D is inverted or whether there is an insertion of heterochromatin (without inversion) between 88D and 21E. The tip of 3R to 88D is exchanged for the tip of 2L.

In-ho—Inversion (*2*) *heldout.*—LEWIS 1940. *In/ho* is phenotypically like homozygous *ho*. Opposite *Df-S3*, *S* or *ast*, the inversion acts as though it carries the normal alleles of *S* and *ast*. The homozygote has reduced eyes with anterior indentation; the wings are reduced to tiny stubs (*In/vg* is wild type); the female is fertile, but the male lacks genitalia and the anal apparatus. The break points of this short inversion occur just to the left of the 21E1–2 doublet and just to the right of the 22E1–2 doublet.

T(2;4) ast^V—Translocation (2;4) asteroid-variegated.—LEWIS 1940. *ast^V/+* is wild type; *ast^V/ast* and *ast^V/S* resemble but are more variable than *ast/ast* and *S+/+ast*, respectively. *ast^V/Df-S3* is lethal. *ast^V/ci* gives a cubitus interruptus (*ci*) effect. The variegated *ast*- like effect is completely suppressed in the XXY female. Duplication and deficiency derivatives are viable. *Df-ast^V/+* resembles *Df-al/+* except that the former has a much more roughened eye; the venation of *Df-ast^V/net* resembles homogygous net; *Df-ast^V/l-gl* is lethal. *Dp-ast^V/l-gl/l-gl* is viable. One break just follows the 21E1–2 doublet and the other is in the heterochromatin of chromosome 4.

Df-S7-Deficiency (2) Star-7.—LEWIS 1940. From X-rayed *net ho/net Dp-S sp cl* male. Associated with *net* and *ho*. Loss extends from just after the medium 21C3 band to just preceding the heavy 22A1–2 doublet. Lost.

With this material it was possible to locate with varying degrees of precision most of the known mutants at the extreme left end of the second chromosome. The results are diagrammed in figure 1. The locations of *al*, *ex*, and *shr* are based directly on tests of these mutants to the *S* deficiencies and *Df-al*. It is likely that the locus of *ho* is in the neighborhood of section 22E on the basis of genetic and cytological studies of *In(2)ho*. The location of *l-gl* follows from studies of the duplication and deficiency derivatives of *T(2;4) ast^V* which "cover" this locus in contrast to the *S* deficiencies and *Df-al*.

Net (0.0—)

The process of locating the *net* locus was complicated by the enhancement of *net* by Minute deficiencies of the *Df-al* and *Df(2)ast^V* type, and by the possible complete suppression of the *net* phenotype by the *S* effect of the *S* deficiencies. The latter possibility was excluded by the fact that *net Df-S7/net* flies had only a very slightly suppressed *net* venation. Critical evidence for the location of *net* was obtained by the use of a duplication for the left end of chromosome 2 derived from *T(2;4)b* of DOBZHANSKY. Females of composition *T(2;4)b, net/net* were crossed in separate experiments to *Df-al/Cy* and *Df-S5/Cy* males. The non-Curly duplication offspring having the composition *Dp(2)b, net/net/Df-al* and *Dp(2)b, net/net/Df-S5*, respectively, had normal venation. On the other hand, when females of the above type were mated to *T(2;4)ast^V/Cy* males, some of the duplication offspring had typical *net* venation and therefore presumably had the genetic make-up *Dp(2)b, net/net/Df(2)ast^V*. These results are consistent with the fact that *Df(2)ast^V/net* flies show a more extreme *net* venation than *Df-al/net* flies. Hence the locus of *net* must lie to the left of the 21C1–2 doublet.

The locus of *net* (originally 0.3± on the genetic map) was reexamined, but the results were negative with respect to whether *net* is to the left or to the right of *al*. Thus, on the basis that it is to the right of *al*, no crossovers were obtained between the locus of *al* and *net* among 90 tested crossovers between *al* and *S*. Again, on the basis that it is to the left of *al*, there were no crossovers between *net* and *al* among ten tested crossovers between *net* and *S*. From the cytological evidence, however, it is likely that the *net* locus is to the left of

al. It should be noted here that the map order of *net* (0.0—) and *l-gl* (0.0—) is unknown.

As has already been mentioned, (+*as*')(+*ast*) *ho/net S dp cl* females produced a very rare type having the composition, *ast ho*. A total of all matings in which females of this type were used produced three *ast ho* to 247 *net ast ho* crossovers, the latter occurring with a frequency of 0.35 per cent. The *ast ho* types, which were normal cytologically, were first interpreted as indicating that the locus of *net* was included in the region duplicated in *Dp-S* (LEWIS 1941). This tentative conclusion is now no longer consistent with the deficiency evidence for the location of *net*. The *ast ho* types may have resulted from double crossing over, which is very improbable for a region only one to two map units in length, or from unequal crossing over involving sister chromatids of the duplication chromosome.

Dachsous (0.3)

The deficiencies, *Df-al*, *Df-S2*, and *Df-S6* (identical with *Df-S2*, but independent in origin) appear to be deficient for the *ds* locus as determined by tests of these deficiencies not only to the mutant ds^1, but also to ds^W, ds^k, and ds^{38k}. Yet, cytologically the above *S* deficiencies do not appear to overlap *Df-al* (fig. 1). In the case of both *Df-S2* and *Df-S6* it is possible that the loss involves only the right half of the 21D1–2 doublet; in any case, they are separated from *Df-al* by at least one disc of the chromosome. Perhaps the simplest explanation of these ambiguous results is that either *Df-al* or *Df-S2* (and *Df-S6*) is a true deficiency for the *ds* locus while the other has a position effect at, or an interaction with, the *ds* locus resembling deficiency for that locus.

Star (1.3)

Purely genetic evidence has shown that the *S* locus is included in the repeated section of *Dp-S*. That evidence has included not only the "extraction" of the normal allele of *S* from each section of (+*ast*)(+*ast*) but also the "insertion" of *S* itself into each section. Hence, the *S* locus must lie in the region from 21D3 to 21E1–2, inclusive—that is, the region present twice in *Df-S*. This shows that the deficiencies, *Df-S1* to *Df-S7*, inclusive, which have in common a loss of that section and which closely resemble *S*, phenotypically, are indeed deletions for the *S* locus.

A more precise location of *S* can be made with the use of $T(2;4)ast^V$ and *T*(*Y*;2)*21E*. The latter translocation was kindly supplied by DR. SCHULTZ, who found (unpublished) that the second chromosome was broken just to the left of the 21E1–2 doublet and translocated with the Y chromosome. *T*(*Y*;2)*21E* over *S* or *ast*, acts as though it carries the normal alleles of *S* and *ast*. The deficiency for the tip of chromosome 2 derived from this translocation closely resembles *Df-al* in its effects. Whereas $Df(2)ast^V/S$ is lethal, *Df*(2)*21E/S* is very similar to $Df(2)ast^V/+$. This suggested that the loss of the 21E1–2 doublet is associated with an *S* effect. To eliminate as far as possible the effects

of deficiencies for euchromatic regions other than the 21E1–2 doublet, these two translocations were combined to produce a derivative having the tip of chromosome 2L up to and including 21D4, derived from *T(Y;2)21E*, and the remainder of the second chromosome, from 21E3 on derived from $T(2;4)ast^{V}$. For this purpose *y/Y* (attached-X): *T(Y;2)21E/Cy, E-S* females were mated to $T(2;4)ast^{V}/Cy$, *(2L)dp² b pr* males. F_1 Curly flies with eyes resembling those of *S/E-S* were mated *inter se* to establish a balanced stock of this newly derived deficiency. An examination of the salivary gland chromosomes of this stock showed that the expected combination of the two translocations was realized. This synthesized deficiency has been called Deficiency (2) Star-derived, *Df-S-der*. As kept in stock, *Df-S-der* has a deficiency for the 21E1–2 doublet, a duplication for part of the Y chromosome, and a deficiency in the heterochromatin of chromosome 4. Phenotypically, *Df-S-der* was indistinguishable from the other *S* deficiencies in its effects opposite +, *S*, *ast*, ast^{2}, and ast^{4}. Thus, the *S* locus is closely confined to the region of the 21E1–2 doublet structure of the salivary gland chromosomes.

Asteroid (1.3+)

It is well recognized that the determination of genetic and cytological correspondences by deficiency evidence alone may sometimes be misleading, owing to the possibility of position effects accompanying these deficiencies. As has already been described, such difficulties were encountered in attempting to ascribe the *ds* locus to some precise section of the chromosome. In view of the position effect known to exist at the *S* and *ast* loci, it is apparent that these loci present a special problem. In the case of *S*, its location by means of deficiencies could be independently checked by the use of a tandem duplication for that locus—namely, *Dp-S*. Similar methods have been used in the location of *ast*.

It can be safely assumed that *Df-S4*, which involves a loss of the shrunken locus, is a deficiency for the *ast* as well as the *S* locus. Moreover, it more closely resembles *S ast* than $S\ ast^{+}$ in its effects opposite *ast*. Inasmuch as *Df-S-der* is phenotypically indistinguishable from *Df-S4*, it is likely that it too involves a loss of both the *S* and *ast* loci. It is important to note here explicitly that *Df-S-der* is derived from *T(Y;2)21E*, which has no detectable departure from normal when opposite *S* or *ast;* and from $T(2;4)ast^{V}$ which likewise behaves like wild type when the variegation is suppressed by an extra Y chromosome. Now it has been determined that the presence of an extra Y chromosome does not change the phenotypic effects of *Df-S-der*. It may therefore be assumed that those effects are attributable mainly to the deficiency for the 21E1–2 doublet.

Considerable evidence is available for supposing that the *ast* locus, like the *S* locus, is included in *Dp-S*. This evidence will be described in a later section and comes from a further study of the position effect at the *S* and *ast* loci by means of chromosomal aberrations and by means of various derivatives of

Dp-S. Formal genetic proof that the *ast* locus is included in *Dp-S* is lacking, however, since the occurrence of the crossover between an asteroid locus in the left section of the duplication and the break point of the duplication has not been definitely detected.

Suppressor of Star

The dominant Suppressor of Star, *Su-S*, found by CURRY, is mentioned here since it has been reported to be close to, if not an allele of *S*. A reinvestigation of *Su-S* has shown that it is probably not the result of a point mutation but rather that its effect is attributable to the double deficiency involved in *In(2L)Cyt*, with which it was associated at the time its effect was first detected. This was shown by deriving, anew, *In(2L)Cyt*, from a mating of *In(2L)Cy*, $dp^2/In(2L)t$ females to *S dp/Cy* males. *In(2L)Cyt* was detected in the progeny of this cross by virtue of its suppressing effect on *S*. The complementary or double duplication derivative was also detected in this experiment by virtue of its slight enhancement of *S*. A cytological analysis of *In(2L)Cyt*, made by BRIDGES and LI (1936), showed the presence of a deficiency for 22D3 and all of section 34A. There is some evidence of a preliminary nature for supposing that it is the deficiency in 22D which is responsible for the suppression of *S* by *In(2L)Cyt*. Whether or not the location of Enhancer of Star, *E-S*, (locus, 2– 6±) coincides with that of *Su-S* is not known. Existing stocks of *E-S* that have been examined cytologically show the presence of the Curly inversion and not the double duplication derivative complementary to *In(2L)Cyt*. The enhancement of *S* by this duplication type is by no means as extreme as that by *E-S*.

THE PHENOTYPES OF DP-S DERIVATIVES

Unequal crossing over provides a mechanism whereby different *S* and *ast* alleles can be introduced into *Dp-S*. It seemed likely that a study of the derivatives, so obtained, should shed further light on the nature of position effect at these loci. Using only the following six combinations of *S* and *ast* alleles: ++, $+ast^4$, $+ast$, $S+$, $S\ ast^4$, and $S\ ast$; there are 36 possible ways of varying the composition of *Dp-S*. Actually, it was not feasible to vary the composition of the *ast* locus in the left section of the duplication. This leaves twelve combinations, of which all but two, $(+ast)(+ast^4)$ and $(S\ ast)(+ast^4)$, have been synthesized. The derivatives may be divided into the following two groups:

Group I	Group II
1. $(+ast)(++)$	6. $(S\ ast)(++)$
2. $(+ast)(+ast)$	7. $(S\ ast)(+ast)$
3. $(+ast)(S+)$	8. $(S\ ast)(S+)$
4. $(+ast)(S\ ast)$	9. $(S\ ast)(S\ ast)$
5. $(+ast)(S\ ast^4)$	10. $(S\ ast)(S\ ast^4)$

In each of these ten types the presence of the duplication was confirmed by an examination of the salivary gland chromosomes. Only a brief description

will be given of the methods by which these types were originally obtained.

All members of Group I were obtained from (+*ast*)(+*ast*). The unequal crossover, *net* (+*ast*)(++) *ho*, was detected among the progeny of *net* (+*ast*)(+*ast*) *dp cl/al ho* females as a complete suppressor of S^2 *E-S* (*Cy*); whereas, (+*ast*)(+*ast*) only partially suppresses S^2 *E-S*. The derivatives 3, 4, and 5 could be obtained with relative ease from (+*ast*)(+*ast*)/*S*, (+*ast*)(+*ast*) /*S ast*, (+*ast*) (+*ast*)/(*S* ast^4) females. respectively, when mated to *S/Cy* males. The marker genes are omitted here for simplicity. The new combinations, 3, 4, and 5, when opposite *S*, are found to be inseparable in appearance from *S*/+; while (+*ast*)(+*ast*)/*S* has a normal or only very slightly roughened eye.

A brief description has already been given of the method of deriving (*S ast*) (+*ast*) from (+*ast*)(+*ast*). Because of the difficulty of introducing *S* into the

TABLE 7

Phenotypic effects of certain Dp-S heterozygotes. The composition of the duplication chromosome is shown at the left while that of the normal chromosome is shown at top. The entries in the table indicate standard diploid types to which the appropriate Dp-S heterozygotes are very closely comparable.

		+	*ast*	*S* ast^4	*S ast*	*S*	*Df-S3*	*Df-S-der*
1	(+ *ast*)(+ +)	"+"	"+"	"+"	"+"	"+"	"+"	"+"
2	(+ *ast*)(+ *ast*)	"+"	"+"	"*ast*/+"	"*ast*/+"	"*ast*/+"	"*ast*/+"	"*ast*/+"
3	(+ *ast*)(*S* ast^4)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
4	(+ *ast*)(*S ast*)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
5	(+ *ast*)(*S* +)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
6	(*S ast*)(+ +)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
7	(*S ast*)(+ *ast*)	"*ast*/+"	"*ast*"	"*S* ast^4/*ast*"	"*S ast*/*ast*"	"*S*/*ast*"	"*S ast*/*ast*"	"*S ast*/*ast*"
8	(*S ast*)(*S* ast^4)	"*S*/+"	"*S* ast^4/*ast*"	lethal	lethal	lethal	lethal	lethal
9	(*S ast*)(*S ast*)	"*S*/+"	"*S ast*/*ast*"	lethal	lethal	lethal	lethal	lethal
10	(*S ast*)(*S* +)	"*S*/+"	"*S*/*ast*"	lethal	lethal	lethal	lethal	lethal

left section of *Dp-S*, and of detecting the product when it is produced (*S ast*)(+*ast*) was used as the source of the remaining members of Group II. This, then, was accomplished by methods similar to those by which members of Group I were derived from (+*ast*)(+*ast*) except that the detection of the Group II derivatives was on a different basis. One example will be given to illustrate this difference. Females of composition *al* (*S ast*)(+*ast*)/*net S dp cl*, were mated to *al ho* males. F_1 *al* "*S*/+" offspring were then outcrossed to an *al ho* stock, and the F_1 larvae from this latter mating were then analyzed cytologically for the presence of *Dp-S*. It was found in this way that roughly one-half of the *al* "*S*/+" crossovers had carried the duplication; in other words, they were of the expected type, *al* (*S ast*)(*S*+). Similar methods had to be used for each of the remaining members of Group II.

The phenotypic effects of ten *Dp-S* derivatives when opposite +, *ast*, *S* ast^4, *S ast*, *S*, *Df-S3*, or *Df-S-der*, are shown in table 7. Those types which have a normal eye or occasionally a very slightly roughened one are compared with *ast*/+, to which they are perhaps most similar. Although a deficiency for, and only for, the region present twice in *Dp-S* is not available, an approximation can be realized by comparing the effects opposite *Df-S-der*, and *Df-S3*, whose

deficiencies are respectively smaller and larger than that region. From table 7, it is apparent that these deficiencies act alike in all combinations shown. Two important combinations of these deficiencies are those with (+*ast*)(*S*+) and (*S ast*)(++). In each case the effect is more nearly similar to that of *S*/+ than to *Df-S*/+. The effects of these two deficiencies when opposite (*S ast*)(+*ast*) are compared with those of *S ast*/*ast*; they might equally well be compared with *ast*/*Df-S*.

It has already been reported that types analogous to double-Bar and quadruple-Bar have been obtained from *Dp-S* (LEWIS 1941). The triple form, since it arose from (+*ast*)(+*ast*) females by unequal crossing over, has the composition $(+ast)_3$. The subscript, 3, here is used to indicate that the section present in parenthesis occurs three times in tandem repetition. Using a similar notation, the quintuplication, which was derived from homozygous $(+ast)_3$ females, has the composition $(+ast)_5$. A comparison of $(+ast)_2/(+ast)_2$ and $(+ast)_3/ast$ shows no detectable difference. That is, the eye looks normal in each case, and slight extra veins are occasionally present in each. No (detectable) position effect was found in a comparison of $(+ast)_3/(+ast)_3$ with $(+ast)_5/ast$. Each of these latter types has an eye slightly larger than normal with bulging and somewhat disrupted facets; extra veins are also present in each to apparently the same extent.

Experimental evidence for the nature of the position effect in (+*ast*)(+*ast*) has been derived from a study of a translocation, *T(2;3) Dp-S*, obtained from X-radiation of (+*ast*)(+*ast*) males. A salivary gland chromosome analysis showed the presence of a reciprocal translocation, having one of its breaks within the duplication, just to the left of the 21E1–2 doublet of the right section of *Dp-S*, and the other break in heterochromatin of 3R. In the presence of a normal heterochromatin balance, the phenotypic effects of *T(2;3)Dp-S* are such as might be expected if it contained two doses of *ast*, just as the notation (+*ast*)(+*ast*), would indicate; thus, *T(2;3)Dp-S/S* is similar to, although more variable than, homozygous *ast*. However, when studied in the XXY female, this latter combination has a normal eye, or occasionally a slightly roughened one, typical of that of (+*ast*)(+*ast*)/*S*. In other words, the effects of (+*ast*)(+*ast*) appear to be the same whether the *S ast* regions are relatively close, as in *Dp-S*, or are widely separated, as in *T(2;3)Dp-S*. It is important to note here explicitly that the phenotypic effects of (+*ast*)(+*ast*), itself, are not changed by the addition of a Y chromosome.

X-ray induced changes at the S and ast loci

An analysis of X-ray-induced changes at the *S* and *ast* loci offers another line of attack on the nature of the position effect at these loci. Such a study must be limited by the practical difficulty of obtaining a sample of such changes without employing their phenotypic effects as a basis for their detection. In the experiment shown in table 6 it was possible to detect changes from wild type which closely resembled either *S* or *ast*. Neglecting deficiencies, a change of each type was detected—namely, $T(2;3)S^L$ and $T(2;4)ast^V$. To these aberrations should

be added the *S* translocation of MULLER (MULLER and PAINTER 1929), *T(2;3)S*^{M}, which likewise arose from wild type. The latter *S*, however, was detected by its dominant *S*-like effect opposite wild type. In spite of the differences in the bases for detection of *S*^{L} and *S*^{M}, the phenotypic effects of these changes, as far as could be determined by tests to various *S* and *ast* alleles, were identical with that of *S* itself. The addition of an extra Y chromosome did not alter the phenotype of either *S*^{L} or *S*^{M}. The recessive change, *ast*^{V}, was more similar to *ast*^{1} than the other *ast* mutants in its effects on the venation and eyes.

Cytologically, *S*^{M}, *S*^{L}, and *ast*^{V} have in common a break immediately following the 21E1–2 doublet, as indicated in figure 1. In this connection, one other aberration obtained from wild type, *In(2)ho*, also has a break at this point. As already noted, *In(2)ho* does not appear to be associated with a change at the *S* or *ast* loci. Similarly, *T(Y;2)21E* has no detectable effect at these loci. Its break in the second chromosome, however, is immediately to the left of the 21E1–2 doublet.

Only one change at the *S* and *ast* loci not associated with a demonstrable rearrangement in the salivary gland chromosomes occurred in the experiment shown in table 6. This change has been given the tentative symbol, *S*^{X} *ast*^{X}, since, as shown below, it behaves genetically as though it involves a simultaneous change at each locus. *S*^{X} *ast*^{X}/+ flies show an occasional gap near the tip of the second longitudinal vein and have slightly smaller eyes than those of S/+ flies. It was found that if these slight differences from *S* are due to modifiers, those modifiers must be very closely linked to *S*^{X} *ast*^{X}. Opposite the *ast* alleles, *ast*, *ast*^{2}, *ast*^{3}, amd *ast*^{4}, *S*^{X} *ast*^{X} closely resembled *S ast*$^{+}$.

On the assumption that *S*^{X} *ast*^{X} represented merely a new dominant allel of *S* a search was made for "wild type" crossovers from *S*^{X} *ast*^{X}/*ast*^{4} females similar to those obtained from *S*/*ast*^{4} females. In this experiment females of composition *In(1)dl-49*, *cm*^{2}/+; *S*^{X} *ast*^{X}/*ast*^{4} *ho*; *In(3LR)sep*/+, were individually mated to *al S ho*/*Cy*, *E-S* males. This mating produced, among 3,505 non-Curly offspring, a single female resembling the expected wild type class. The latter fly had normal (non-*ho*) wings, but the eyes were slightly smaller than those of *S*/+. Upon further testing, the crossover chromosome appeared to carry a very slight *ast* allele, *ast*^{X}. It is of course possible that *ast*^{X} arose in the above experiment as a mutation of *ast*^{4}. The following properties of *ast*^{X} were determined: *ast*^{X}/+ and *ast*^{X}/*ast*^{X} are wild type; *S*/*ast*^{X} closely resembles *S*^{X} *ast*^{X}/+ except that the former frequently, if not always, has normal venation; *S*^{X} *ast*^{X}/*ast*^{X} has a somewhat smaller eye size than that of *S*^{X} *ast*^{X}/+. A salivary gland chromosome analysis of *ast*^{X}, as well as of *S*^{X} *ast*^{X}, failed to show any disturbance in the left end of the second chromosome. The possible significance of the *S*^{X} *ast*^{X} change will be taken up in the discussion of this paper.

It will be recalled that the spontaneous *S* duplication, *Dp-S*, has a break immediately to the right of the 21E1–2 doublet (of the left section of the duplication), similar to that found in *S*^{L}, *S*^{M}, and *ast*^{V}. Phenotypic studies of *Dp-S*

suggested that the *ast* mutant originally present in the left section of the repeat acts as though it had reverted to wild type. With this result particularly in mind, *al ast ho/net ast dp cl* males were X-radiated (6,500 r units) and mated to *S/Cy, E-S* females. "Reversions" of *ast* were looked for among the F_1 flies. The following three changes closely resembling reversions of *ast* to *ast*+ were found, all of which were fertile:

T(2;3) ast^{rv1}—Translocation (2;3) asteroid reverted-1.—LEWIS 1942. The translocation carries the mutants *al* and *ho*. Phenotypically, *ast^{rv1}* is inseparable from wild type opposite +, *ast*, *ast^3*, *ast^4*, *S ast$^+$*, *S ast*, *S ast^4*, *E-S*, and *ast^V*. The homozygote is lethal. However, *ast^{rv1}/Df-S4* is viable and resembles *S/*+ rather than *Df-S4/*+. The eye is slightly less rough in either *ast^{rv1}/S^M* or *ast^{rv1}/S^L* than it is in *S^M/*+ or *S^L/*+. At least three breaks are involved in this complex rearrangement. Chromosome 2L is broken just to the right of 21E1–2; chromosome 3L has a break just after the heavy dotted 68C2 band and 3R has a break which probably just precedes 88D9 (BRIDGES' 1935 map). The new rearrangement is in the form of cyclical exchange of tips. Thus, 2L tip replaces the tip of 3R, which in turn replaces the 3L tip, which in turn replaces the tip of 2L.

In(2L)ast^{rv2}—Inversion (2L) asteroid-reverted-2.—LEWIS 1942. The inverted chromosome also carries the mutants, *al* and *ho*. Phenotypically, *ast^{rv2}* is identical with *ast^{rv1}*, including its effects opposite *Df-S4* and *S^L*. *S^M/ast^{rv2}* overlaps wild type. *ast^{rv1}/ast^{rv2}* is wild type. A preliminary salivary gland chromosome analysis showed a break immediately following the 21E1–2 doublet and one in section 31, the section between then being inverted.

T(2;3) ast^{rv3}—Translocation (2;3 ast^{rv3}).—LEWIS 1942. The translocation was associated at the time of its origin with the mutants, *net*, *dp*, and *cl*. The homozygote and *ast^{rv3}/Df-S4* are lethal. Incomplete phenotypic studies indicate that opposite *S*, *S ast*, *S ast^4*, and *S^L*, *ast^{rv3}* produces a slightly rougher eye than that characteristic of *S/*+. *ast^{rv3}/ast^3* and *ast^{rv3}/E-S* are wild type. The rearrangement involves a reciprocal exchange of the extreme tips of chromosomes 2L and 3L, with breaks just to the right of 21E1–2 and to the left or right of 61C1. In its new location this latter region of chromosome 3L assumes a heterochromatic character.

Again, as in *Dp-S*, apparent reverse mutation of *ast* was associated with a chromosomal rearrangement in which the 21E1–2 doublet was transferred to a new position. As yet, no spontaneous or induced reverse change of *ast* to *ast$^+$* unaccompanied by a chromosomal rearrangement has been detected.

The above results further support the conclusion that *ast* as well as *S* is included within the confines of the 21E1–2 doublet, for it is this doublet and not the material to the right of it which has undergone rearrangement in both *Dp-S* and the X-ray induced asteroid-reverted changes. That is, in *Dp-S* the material to the right of 21E1–2, since it not present twice, is in its normal position following the 21E1–2 doublet of the right section of the repeat.

From the same X-radiation experiment which produced the asteroid-re-

verted changes, a complete suppressor of *S* was detected as a single (sterile) male with normal eyes and wings. Several changes resembling partial reversion of *ast* were also detected. Among those of the latter which were analyzed, two were found to be due to permanent changes. They were complex rearrangements in which the 21E region was normal, but each had in common a break in or close to section 22D and had that section translocated to heterochromatin of chromosome 3. It is possible that the partial suppressing effect on *S/ast* of these aberrations is related to that shown by the double deficiency *In(2L)Cyt* or *Su-S*, which has one of its deficiencies in 22D.

DISCUSSION

A plausible interpretation of the *S* and *ast* loci can be developed by assuming that they have resulted from duplication of an ancestral locus, that duplication now being established in the species. This notion is chiefly based on the finding that *S* and, very probably, *ast* are included in the 21E1–2 doublet structure of the salivary gland chromosomes. The possibility that such structures might represent instances of duplication of a single band, or short section, was first pointed out by BRIDGES (1936). It is interesting to note that BRIDGES chose the 21E1–2 doublet as a characteristic example of this type of duplication.

That the doublets involve two discrete bands was shown experimentally by BRIDGES et al. (1936) in an analysis of the Notopleural deficiency. In this case the break points of the deficiency occurred between the halves of two doublets. BRIDGES (Drosophila Information Service **9**) has reported other instances, chiefly spontaneous, where breaks have separated the halves of a doublet. Similarly, METZ (1937) has found that some of the small deficiencies which occur in stocks of *Sciara ocellaris* involve the loss of only one band of a doublet structure. METZ (1938) also holds to the view that doublets have arisen by a process of duplication and, on this basis, that these "deficiencies" are either the original unduplicated condition or are secondary losses from duplications already established in the species.

Small, intrachromosomal duplications have been given the convenient name "repeat" by BRIDGES. The doublet structures fall in the category of "tandem repeats" in which the duplicated section immediately follows (or precedes) the original section. The tandem repeat may be either "direct" or "reverse." Thus, if the section present twice in the repeat is represented by the letters, CD, then a direct tandem repeat can be written as CDCD, and a reverse tandem repeat as CDDC or DCCD. The direct repeats such as Bar, or the Star Duplication, are unstable in the sense that they can give rise by unequal crossing over to the unduplicated condition, CD, or to a complementary triplicated form, CDCDCD. Reverse repeats, however, are expected to be stable in the sense that unequal crossing over (if it occurs at all) should result only in a dicentric chromatid, which will be lost to the polar bodies, and an acentric fragment.

The problem arises as to which of these two categories of repeats the 21E1–2 doublet may be assumed to belong. This cannot be decided from cytological

evidence alone, since only the duplication of one disc is involved. *A priori*, only the stable or reverse type would be expected to become established in the species. This is supported by the fact that all of the tandem repeats, which have been described for the normal salivary gland chromosomes of *Drosophila melanogaster* and in which the sequence can be determined, are of the reverse type. Moreover, if the 21E1–2 doublet were a direct repeat, then it might be anticipated that some of the changes observed at either the *S* or *ast* loci would represent either the unduplicated or the triplicated forms. However, no cytological changes whatsoever in the 21E1–2 doublet were apparent in any of the mutants at these loci or in any of the analyzed crossovers between these loci.

Assuming from the above considerations that the 21E1–2 doublet is a tandem reverse repeat, we can draw the genetic analogy that *S* and *ast* are reversely repeated loci. There are at least two methods by which reverse repeats may offer a basis for producing changes which resemble mutation but which are due to crossing over in the repeat. Firstly, genetic differences in the duplicated section of a naturally occurring repeat may arise within a stock or between different stocks. Such differences need not be detectable, phenotypically, since the repeated section is present in four doses. Applied to the *S* and *ast* loci, this mechanism suggests that such differences as are observed between $S+/+ast$ and $S\ ast/++$, or between $S+/+ast^4$ and $S\ ast^4/++$, may be due, not to position effect, but to unknown genetic differences in what were thought to be normal alleles. However, this is very unlikely in that the "wild type" crossovers derived from either $S+/+ast$ or $S+/+ast^4$ females are identical not only with each other but also with normal chromosomes from unrelated stocks, even in the critical test opposite $S\ ast$ or $S\ ast^4$. In particular, the critical test held in an experiment in which the "wild type" crossover and $S\ ast^4$ were each recovered in the same experiment from $S+/+ast^4$ females.

Secondly, consider a tandem repeat in which the two halves are no longer exactly identical, genetically, and assume that an inversion of the repeat has been produced. The original repeat may be written CDD′C′, where the primes indicate genetic differences between the repeated regions. For simplicity, the inversion may be assumed to involve only the repeat itself and may be represented as C′D′DC. It is apparent that this simple case has a highly important consequence—namely, that the inversion cannot be detected cytologically. Crossing over in CDD′C′/C′D′DC heterozygotes will give rise to genetically different products. For example, if crossing over occurs between D and D′, then these products are CDDC and C′D′D′C′. This mechanism is suggested as a possible basis for the behavior of the X-ray induced *S*-like change, $S^X\ ast^X$, which from linkage studies appeared to involve a change at the *ast* locus as well as the *S* locus. Here it is necessary to assume that the *X*-rays induced an inversion of just the repeated region, since $S^X\ ast^X$ was normal, cytologically. The new sequence may be written $ast^+\ S^+$, and the *S*-like effect may then be attributed to a position effect rather than to a point mutation. On this basis, the "mutant" ast^X, recovered from $S^X\ ast^X/++$ females may be interpreted as a duplication for the *ast* locus and a deficiency for the *S* locus—that is, its

composition may be written ast^+ ast^+. This mechanism does not seem applicable to the spontaneous *S* and *ast* mutants, since the crossover corresponding to ast^+ ast^+ does not differ detectably from S^+ ast^+; furthermore, S^+ could not be here assumed to be identical with ast^+, for then the inversion itself would accomplish nothing new.

The concept of *S* and *ast* being repeated loci is also useful in that it relates, by analogy, the position effect observed at these loci to that occurring at a known repeat region—namely, the Bar Duplication. As a result of the cytological finding of BRIDGES (1936) and MULLER, et al. (1936) that Bar is a duplication, the position effect demonstrated by STURTEVANT (1925) may now be stated as depending on the way in which four, rather than two, homologous sections are distributed between the two homologs, Thus, equal distribution of the four doses of the Bar region results in a larger eye than when three doses are present in one homolog and the fourth in the other. The position effect, here, would appear to extend over a distance at least as great as the length of the duplication, or perhaps as great as the length of triplicated region. This is remarkable in view of the fact that other types of rearrangements which involve only euchromatic portions of the chromosomes and which seem to be accompanied by position effects do not show this spreading effect.

It is suggested that this spreading effect is due not alone to the closeness of the two elements in Bar, or the three elements in *BB*, but likewise to the homology of those elements. This possible role of repeats in producing pronounced position effects will be discussed more fully in connection with position effects at the *S* and *ast* loci.

Several methods of attack were used in studying position effect at these loci. Firstly, their effective position could be altered by allelic substitutions alone. Thus, no demonstrable chromosomal aberrations appeared to accompany *S*, *ast*, ast^4, *S* ast^4, or *S ast;* yet, a striking difference exists (1) between *S*/*ast* and *S ast*/+, and (2) between *S*/ast^4 and *S* ast^4/+. Similarly, *S ast*/ast^4 is genetically equivalent to *S* ast^4/*ast;* yet the former has a larger eye and less interrupted wing venation than has the latter. It may be emphasized that in comparisons (1) and (2) there is an important qualitative difference in that neither *S ast*/+ nor *S* ast^4/+ has ever been observed to overlap the small, roughened eye types characteristic of either *S*/*ast* or *S*/ast^4; whereas, *S ast*/ast^4 and *S* ast^4/*ast* may occasionally resemble one another.

This position effect at the *S* and *ast* loci may be stated more analytically. The viable, diploid combinations of *S*, ast^4, *ast*, *S* ast^4, *S ast*, and wild type can be arranged in the following series (the symbol, >, means " has a larger and less roughened eye than," while the symbol, =, means " is phenotypically approximately equivalent to"): $+/+ = ast^4/+ > ast/+ > ast^4/ast^4 > S\ ast^4/+ = S\ ast/+ = S/+ > ast/ast^4 > S\ ast^4/ast^4 > S\ ast/ast^4 > S/ast^4 = ast/ast > S\ ast^4/ast > S\ ast/ast > S/ast$. From this seriation it may seen that if (n) represents any one of the six combinations of these alleles, then the order may consistently be written: $+/n > ast^4/n > ast/n > S\ ast^4/n \geqq S\ ast/n \geqq S/n$. Here, the order, $S\ ast^4/n > S\ ast/n > S/n$, is determinate only when (n) is *ast* or ast^4.

The above analysis shows that the order of effectiveness of the *ast* alleles is different, depending on whether they are adjacent to S^+ on the one hand or to *S* on the other. Thus, when these alleles are adjacent to S^+, their effective order may be written: $+ > ast^4 > ast$; while adjacent to *S*, it becomes: $ast^4 > ast > +$. The difference between *S* ast^4/ast and *S* ast/ast^4 leads to the conclusion that more than just a change in the order of effectiveness of the *ast* alleles is involved; however, the above change of order is another way of stating the pronounced position effects detected in comparisons (1) and (2). The problem arises as to which, if any, of these six haploid combinations of *S* and *ast* alleles does not exhibit a (detectable) position effect. (In this discussion the term, position effect, when applied to the haploid chromosome, is meant to imply an interaction between two, or more, neighboring genes or their products, which leads to a different phenotype than would arise if the genes were widely separated from one another.) As yet, no solution to this fundamental problem is available, since it has not been possible to study the effects of one locus independently of the other. Although none of the available aberrations which have breaks in the vicinity of *S* and *ast* appear to separate these loci, or to involve a loss of one locus and not the other, the effects of these aberrations do have a bearing on the above problem. The study of these aberrations constitutes a second attack on position effect at these loci.

Exclusive of the *S* deficiencies, the available rearrangements involving the *S* region exhibit two significant features. First, only two of these, *T(Y;2)21E* and *T(2;3)Dp-S*, have a break just preceding the 21E1–2 doublet. It is likely that in the latter as well as the former translocation, there is no detectable effect on the immediately adjacent *S* and *ast* alleles. On the other hand, the remaining aberrations have a break immediately following the 21E1–2 doublet, and all of these, except for *In(2)ho*, are associated with a pronounced effect at these loci. This result parallels that found with certain other loci. Thus, among those rearrangements, which have breaks on either side of the scute locus and which do not involve heterochromatin, only those having a break just to the right of the locus are accompanied by a scute-like change. (For a discussion of this case and its bearing on the evidence for position effect accompanying euchromatic exchanges, see MULLER 1941.) The Bar "locus" is another example. Although little material is as yet available, the wholly euchromatic exchanges having a "Bar" effect, including Bar itself, consistently appear to have a break just preceding the 16A1–2 doublet, which structure is known, particularly from studies of GRIFFEN (1941) and SUTTON (1943) to be associated with the Bar effect.

The second important feature is revealed by a study of the non-variegated rearrangements involving the *S* and *ast* regions—namely, all of the available aberrations except for *T(2;4)ast*^{V} and *T(2;3)Dp-S*, whose effects are changed by the addition of a Y chromosome. The former types are associated either with no change whatsoever at the *S* and *ast* loci, as in the case of *In(2)ho* and *T(Y;2)21E*, or they are acccompanied by an identical type of change, the type itself depending on whether the rearrangement occurred in wild type or in

ast. Thus, the dominant *S*-like changes, S^M and S^L, are the only non-variegated aberrations, originating from wild type, associated with a change at the *S* and *ast* loci. In spite of their completely independent origins, and the different bases on which they were detected, these two changes are indistinguishable from each other in all combinations studied. Similarly, the rearrangements which arose from *ast* and which are accompanied by a change at this locus act like complete reversions of *ast* to wild type. That is, partial reversions, although detectable in this case, were not recovered. In the above category belong the X-ray induced changes, ast^{rv1}, ast^{rv2}, and ast^{rv3}. To these may be added the spontaneous *Dp-S*, which appears to have a change to wild type in the action of the *ast* allele in the left section of the repeat.

The above *S*-like and reverted-like *ast* changes can be related on the basis of a possible identical association of the 21E1–2 doublet region in S^L and ast^{rv1}. Thus, these translocations exhibit a remarkable coincidence in that they apparently have identical breaks in 21E and 68C. Whether the 21E1–2 doublet is intimately associated with 68C in S^L, as well as in ast^{rv1}, is not determinate, owing to the complication of an additional break in heterochromatin in the S^L rearrangement. However, it is likely that there is a causal rather than a chance basis for this coincidence of breaks.

From these considerations, it may be surmised that the same rearrangement which gave rise to an *S* change from wild type, would, if the original constitution had been *ast*, have given rise to a reversion to wild type. The behavior, if the original composition had been *S*, is conjectural. In this connection, however, extensive experiments on the X-radiation of *S* (unpublished) have failed to give reversions of *S* to wild type. Unfortunately, *Dp-S*, which promised to be the most suitable material for attacking this problem, has so far failed to give crossing over between the *ast* locus of the left section and the break point of the duplication. The effects of *Dp-S* combinations in which the left section carries *S ast*, instead of *ast*, tend to indicate that the effects of that section are similar to those of *S ast* (or *S*).

The third method of attack on the *S-ast* position effect makes use of *Dp-S* to obtain combinations equal on a quantitative genetic basis, but involving differences in the distribution of the *S* and *ast* alleles between the two homologs or between the two sections of the repeat. Inspection of table 7 shows that a great many combinations of this type are lethal; such as, (*S ast*)(*S ast*)/*S* and (*S ast*)(*S* +)/*S ast*. In many cases, no difference can be detected between the members—that is, $(+ast)^4(S+)$ cannot be distinguished from (*S ast*)(++) in a great many combinations studied. In certain instances, very sharp differences exist, as in each of the seven comparisons involving the equivalent types, (+ *ast*)(*S ast*) and (*S ast*)(+ *ast*). Superficially, such differences appear to indicate an interaction between the two sections of the repeat—that is, a position effect extending across the repeated regions. A much more likely and consistent assumption is that in those duplication derivatives having S^+ *ast* in their left section, the rearrangement has induced a position effect on these loci causing them to act like S^+ ast^+. There are three reasons for making this

assumption. Firstly, it satisfactorily explains the effects observed for each of the *Dp-S* derivatives which carry *ast* in their left section, without introducing the additional assumption of position effects extending from the *S* and *ast* loci in one section to their duplicates in the other section. Secondly, three other aberrations, ast^{rv1}, ast^{rv2}, and ast^{rv3}, have just such a reverse type of change in the originally present *ast* mutant, and, as in *Dp-S*, this change appears to be a position effect arising from the new association of the 21E1–2 doublet. Finally, the study of *T(2;3)Dp-S* indicated that, at least in the case of $(+ast)_2$, the effects of these two sections are the same whether in close juxtaposition, as in the duplication itself, or are widely separated, as in the translocation.

These conclusions do not necessarily imply that the behavior of *Dp-S* and the *B* duplication are at variance. For it now seems clear that the *B* effect is induced primarily by the new association of the 16A1–2 doublet of the right section of the repeat, rather than to mere duplication of that region, or solely to a position effect between the two sections of the duplication. The difference between *B/B* and *BB/+* indicates that the latter type of position effect does occur; however, it is not clear as to which combination, *B* or *BB*, has the stronger effect in bringing about that difference. Unfortunately, the comparison of $(+ ast)_2/(+ ast)_2$ with $(+ ast)_3/ast$, in which no difference was detected, is not critical, for in neither of these cases does the increase in dosage of the *S-ast* region result in an appreciable change of the eye from normal.

STURTEVANT (1925) and MULLER (1941) have suggested that position effect may be related to the phenomenon of somatic pairing found in the Diptera. This suggestion appears particularly plausible when applied to tandem repeats, since the forces of somatic pairing bring about a very intimate pairing of the duplicated sections within a homolog, and, by so doing, should increase the possibility of the genes in those regions, or the genic products, interacting with one another or competing in a gene reaction. From these considerations, the pronounced position effect existing between the *S* and *ast* loci has been interpreted as a function of their repeat nature and the very short distance between them, both genetically and cytologically speaking. A mutant at one of these loci is viewed as a change in the gene which alters the forces of somatic pairing normally existing between those loci. This possibility is of interest in that it is not necessary to assume that the immediate gene product is altered. The rearrangements which are associated with changes at these loci are likewise considered as upsetting the normal somatic pairing relations between them. It appears that certain relatively non-specific changes in the material just to the right of these loci can cause a considerable change in the position effect normally existing between them.

In general, it may well be found that rearrangements, at least of the wholly euchromatic type, which are associated with position effects, either have a naturally occurring repeat at the basis of those effects or are themselves repeats. *Dp-S* and *B* may be considered as complex types, since they involve a duplication of a doublet structure. The doublets, 21E1–2 and 16A1–2, appear to be responsible for the strong position effects associated with *Dp-S* and *B*, respec-

tively. Superimposed on this position effect is another one which presumably arises from the close juxtaposition of additional doublet regions—a phenomenon as yet detectable only in the case of *B* and/or *BB*.

A repeat interpretation was suggested by OLIVER (1940) for the behavior of two "alleles" of lozenge, lz^r and lz^{g1}, in *Drosophila melanogaster*. This case appears at present to be in many ways similar to that reported here at the *S* and *ast* loci. The alleles, yellow and reddish-alpha, in *Drosophila virilis*, analyzed by DEMEREC (1928), may also be interpreted as duplicate loci, but it is also clear that this alone will not account for the remarkable behavior of the reddish-alpha character. If doublet structures are repeats, as the evidence thus far indicates, then, judging from their widespread occurrence in the salivary gland chromosomes of Drosophila, it is likely that other multiple allelic series may be resolved into duplicate loci which act, by reason of a position effect, as a developmental unit.

SUMMARY

Two loci, Star and asteroid, in the second chromosome of *Drosophila melanogaster* were found to be extremely closely linked, the normal distance between them being estimated as 0.02 map unit.

These loci exhibit a position effect which can be detected solely by varying their genetic composition.

A correlation of genetic and cytological analyses gave evidence that *S* and *ast* are included in the 21E1–2 doublet structure of the salivary gland chromosomes. It also gave pertinent data on the location of other mutants in the vicinity of these loci.

Position effect at the *S* and *ast* loci was also studied by means of chromosomal rearrangements which had breaks in the neighborhood of these loci. A special case was the study of a spontaneous tandem duplication of the Bar type—namely, the Star Duplication, *Dp-S*.

From certain cytological considerations, *S* and *ast* may be interpreted as repeated loci which have become established in the species. The possibility is discussed that repeats have special potentialities for showing pronounced position effects.

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THE GENETIC STRUCTURE OF AMERICAN POPULATIONS OF DROSOPHILA MELANOGASTER

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INTRODUCTION

WILD populations of Drosophila lend themselves admirably to genetic analysis. It is not surprising, therefore, that out of the growing interest of geneticists during the past 15 years in the genetics of natural populations there has come a large amount of literature pertaining to the genetic structure of wild Drosophila populations and to the evolutionary implications of this structure. Particularly notable are the studies of DUBININ and collaborators on *D. melanogaster* populations in the southwestern USSR; of DOBZHANSKY and WRIGHT and their collaborators on western American populations of *D. pseudoobscura;* and of SPENCER and co-workers on *D. hydei, D. immigrans,* and *D. virilis* and its relatives, in this country. The literature from these and other related studies has been reviewed and discussed particularly by DOBZHANSKY (1939, 1941), SPENCER (1940a, 1940b, 1941), MULLER (1940), and TIMOFEEFF-RESSOVSKY (1940).

The present study was begun in 1938 with a view to obtaining comparable data on the genetic structure of several *D. melanogaster* population areas in America. Several brief reports of the work have appeared in abstract form (IVES 1941a, 1941b, 1942, 1943). Further discussions of some of the data appear in PLOUGH, IVES, and CHILD (1939) and in PLOUGH (1941).

Specific questions for which information has been sought for each area include:

1. How much and what kind of genetic variability is present?
2. Is the breeding population continuous in time?
3. Is the breeding population comparatively large or small?
4. What specific factors are influencing the accumulation of genetic variability?
5. What is the probable evolutionary trend in American populations of *D. melanogaster*?

Five population areas have been investigated to some extent. From the data of these investigations it may be said, in general, that American populations of *D. melanogaster* contain an extremely large amount of recessive genetic variability, particularly of a type which causes reduction in viability; that this species appears to breed here in comparatively large populations, continuous in time; that specific genetic and environmental agents appear to influence the accumulation of the genetic variability; and that, within the limits of the evidence of this study, it appears probable that evolutionary change in *D. melanogaster* in this country is taking place at a much slower rate than in the species and populations studied and reported by other authors.

DESCRIPTIONS OF COLLECTIONS

The ideal sample of wild flies for genetic analysis of a given population area is one collected in as small an area as possible and in as short a time as possible, the results of the analysis of this sample to be compared with those of samples from neighboring areas to determine the nature of the population. This ideal has been approximated in the studies of DOBZHANSKY and collaborators and of SPENCER and collaborators. It has been possible to approximate it in only three of the five areas involved in the present study. A description of each collection is therefore desirable.

Two collections were made in a fruit farming district in South Amherst, Massachusetts. The first collection, which appears in the tables of data as Mass. 38, consisted of about 400 flies, mostly old in appearance, which were caught early in October of 1938 in a half-pint milk bottle culture of fly food during a two-day exposure period on the porch of a farmhouse harboring several bushels of decaying fruit. The fruit had not been decaying long enough to culture a generation of flies. These must have flown in from neighboring orchards which, through the late summer months, swarmed with literally hundreds of thousands of flies, but which at this time were nearly barren of both fruit and flies because of fruit harvesting and the first severe frosts of fall. The second collection was made in the same area under similar conditions in September, 1941, but within the course of only a few hours. It appears in the tables as Mass. 41.

Two collections were made in Winter Park, Florida, by PROFESSOR H. H. PLOUGH of this laboratory. The first of these, appearing in the tables as Fla. 40, was made in April 1940. It consisted of decaying fruit from under one tree, the fruit being heavily infested with Drosophila larvae. The flies which developed from these larvae within one week of collection date were analyzed by the author in Amherst. The second collection consisted of adults gathered during the space of a few hours in the same area. These were gathered in March of 1942 and appear in the data as Fla. 42.

PROFESSOR W. P. SPENCER of WOOSTER COLLEGE made a collection for the author in Massillon, Ohio, in September 1941. It appears in the tables as Ohio. SPENCER caught about 500 flies with several sweeps of a collecting net in a large fruit warehouse where he estimated the fly population to have been well over 50,000 individuals. At that time, due to dry weather which had delayed fruit decay, the surrounding orchards contained only a few Drosophila.

DR. MORRIS STEGGERDA of the CARNEGIE INSTITUTION OF WASHINGTON made a collection for the author in Gallup, New Mexico (elevation 6,400 ft.) in September of 1941. He exposed two culture bottles of fly food for a period of two weeks near a restaurant kitchen where he had observed many Drosophila. During this period there were several nights of sub-freezing temperatures. When the cultures arrived in Amherst one of them contained many larvae and pupae and produced 350 flies within a week. The other culture was void of Drosophila. This collection appears in the tables as N. M.

The final collection place was a seashore refuse dump near Belfast, Maine, where PLOUGH exposed four culture bottles for 14 hours in September of 1938.

When brought to Amherst, the cultures contained between them some 25 old appearing flies and many larvae. The larvae need not have been the offspring of the flies which were found in the bottles, since there was nothing to prevent other flies from entering and leaving during the period of exposure. In the laboratory, the old flies and about 200 of the F_1 from the four cultures were put together in a large culture bottle. A random sample was taken from their sons for analysis. This sample consisted of F_1 and F_2 of native Belfast flies. Analysis through the test generation was carried out by PLOUGH. The data appear in the tables as Maine 38. The Maine 38 stock was kept in unselected mass cultures of about 100 pairs of parents for each generation. A random sample of flies was taken from the stock bottle in March of 1942, about 60 generations after the stock was established. It was analyzed by a student, MR. R. SEWARD, under the author's supervision. This second sample appears in the data as Maine 42.

METHODS OF ANALYSIS

The males of each sample of wild flies were analyzed individually by a series of matings designed to reveal the genetic content of one of their two second chromosomes, particularly in so far as this genetic content had any effect on the embryonic, larval, and pupal viability of flies homozygous for these chromosomes. The scheme of these matings is one now well known to geneticists. Each wild male is mated to females from a suitable marker stock, such as *Bl* L^2/*Cy* sp^2, in which one second chromosome is marked by the dominant genes, Bristle and Lobe, and the other second chromosome is marked by the dominant gene, Curly, and in addition carries an inversion in each arm which eliminates nearly all crossing over between the *Cy* chromosome and its homologue. The gene, speck, aids in detecting most of the occasional crossovers which occur. From this mating one male, either *Bl* L^2 or *Cy*, is mated again to *Bl* L^2/*Cy* sp^2 females. F_2 *Cy* flies are then mated for the test generation in the F_3. All non-*Cy* F_3 are homozygous for one of the two second chromosomes carried by the original wild male; and they are expected to appear in the ratio of two *Cy* to one non-*Cy*. If the chromosome carries a recessive gene affecting the phenotype of the homozygote, all the non-*Cy* flies are subject to this effect. If it carries a recessive gene affecting the viability of homozygotes, this will appear as a shift in the ratio of *Cy* to non-*Cy* in the F_3. Where such a viability factor is a lethal gene, all the F_3 are *Cy*, with the exception of an occasional crossover. Other marker stocks used, similar in principle to the above, were *S*/*Cy* sp^2, *S Bl*/*Cy* sp^2, and $M(2)^{40c}$/*Cy* L^4 sp^2.

The kind of genetic variability most easily detected by the above scheme of matings is recessive lethal genes. However, as has been noted by all who have worked extensively with lethals, the completely lethal gene grades into the type that is occasionally non-lethal; and this grades into types that are less and less frequently lethal, until finally one reaches the type that has no lethal effect at all. This situation makes necessary a somewhat arbitrary classification of data concerning genetic factors affecting viability.

After the analysis of the first collection, Mass. 38, was complete, the following system of classification was adopted. This is a system similar in its es-

sential features to that used in the *D. pseudoobscura* studies (DOBZHANSKY and QUEAL 1938). Those chromosomes which failed to appear as homozygotes in a minimum of 200 flies from at least two consecutive test generations of *Cy* by *Cy* matings were classed as lethal. Those which gave between 0 and 17 per cent of homozygotes were classed as semi-lethal. Those which gave between 17 and 30 per cent of homozygotes were classed as deleterious. And those giving over 30 per cent of homozygotes were classed as normal. No lines in any of the collections showed more than 35 per cent non-*Cy* homozygotes. The number of test flies counted for each chromosome line was large enough (average of 500 flies per line) in this first collection to make the difference between the observed percentages of homozygotes in each deleterious line and the normal 33.3 per cent one of statistical significance for all but a very few of the deleterious lines. This was likewise true for the few borderline cases in the deleterious lines of the Fla. 40 collection. In the other collections counts were made from only one test-generation vial for the lines giving over 20 percent of non-*Cy*. This made it impractical to separate them into deleterious and normal classes.

It was observed in all of the collections, as in the *D. pseudoobscura* work, that in most of the chromosome lines there was either less than 10 percent or more than 20 percent of homozygotes in the test generation. Thus, in the combined Mass. 38 and Fla. 40 collections, in only 14 of 378 chromosome lines was there between 10 and 20 percent of homozygotes. Accordingly, the approximate midpoint (17 percent) between lethal and normal percentages of homozygotes conveniently divides the data into two major groups with respect to the influence of the chromosome on the viability of the homozygote. The viability of the homozygotes in the lines showing less than 17 percent of homozygotes is in general so low that all of them may be considered as lethals, without introducing serious error into calculations concerning the genetic structure of the wild populations.

Two methods have been used to determine the statistical significance of differences between means in the data which follow. Standard Error (SE) has been employed when only one degree of freedom has been involved in comparisons and where it was desirable to include this statistic in the tables of data. Chi-square has been used when more than one degree of freedom was involved in a given analysis. These methods have been used to obtain an estimate of the value of p, the probability of obtaining at random as great a variation, or a greater one, between the two or more means under consideration. Statements in the text include only the value of p.

It should be noted further that the SE of the difference between two percentages has been calculated from

$$\sqrt{\frac{p_t \cdot q_t}{N_1} + \frac{p_t \cdot q_t}{N_2}}$$

where N_1 and N_2 are the total numbers of flies upon which the first and second percentages, respectively, are based, and p_t and q_t are the proportions of the

two classes of flies in the total of the two samples, (N_1+N_2). This formula has been used instead of

$$\sqrt{(SE_1)^2+(SE_2)^2}$$

because this latter formula, as pointed out to the author by PROFESSOR MULLER, is true only when $N_1=N_2$, a condition that is seldom realized in most genetical work.

THE OBSERVED GENETIC VARIABILITY

The two collections analyzed in most detail for genetic variability were Mass. 38 and Fla. 40. The data of this analysis are summarized in table 1, where the chromosomes of each collection have been grouped according to their viability

TABLE 1

Genetic variability in wild chromsomes.

COLLECTION	TYPE	*le*	*sle*	*del*	±	TOTAL	% *Cy*+
Mass. 38	-vis	46	3	53	16	118	
	vis	2	17	12	2	33	
	Total	48	20	65	18	151	27.42
							0.22
	%	31.8	13.3	43.0	11.9		
	SE	3.8	2.8	4.0	2.6		
Fla. 40	-vis	100	19	65	4	188	
	vis	0	33	6	0	39	
	Total	100	52	71	4	227	25.14
	%	44.1	22.9	31.2	1.8		0.56
	SE	3.4	2.8	3.1	0.9		

effects and the presence or absence of a visible phenotypic mutation. The viability classification is as follows: lethal (le), semi-lethal (sle), deleterious (del), and normal (+). Within each of these groups is indicated the number of chromosomes with a visible phenotypic mutation (vis) and the number without such a mutation (-vis). Also included is the mean percentage of non-*Cy* flies from the *Cy*×*Cy* test matings of all of the deleterious and normal lines added together (%*Cy*+).

The large amount of genetic variability present in these two population areas is apparent at once in the data of the two collections in table 1. Considering lethals, semi-lethals, and visible mutations, 82 of 151 Mass. chromosomes (54.3 per cent) and 159 of 227 Fla. chromosomes (69.6 per cent) are clearly abnormal. In addition, it seems probable, even without further analysis, that many if not all of the 118 additional chromosomes in the two deleterious groups are sub-normal in viability. Only 9.3 per cent of the Mass. 38 sample and 1.8 percent of the Fla. 40 sample appear to be normal with respect to both viability and phenotypic effects.

The data of table 1 also suggest certain differences in the genetic structure of the Mass. and Fla. populations. There are more lethal and semi-lethal and fewer deleterious and normal chromosomes in the Fla. area. This difference can best be shown by adding the lethals and semi-lethals together in each sample. This is a legitimate procedure, since more than 95 per cent of the lines gave either less than 10 per cent or more than 20 per cent of non-Cy homozygotes. The value of p for the difference between the percentages of lethal-semi-lethal chromosomes in the two samples is less than .001. In addition, the mean percentage of non-*Cy* in the *Cy*×*Cy* matings of the deleterious and normal lines of the two areas suggests that these chromosomes are more dele-

TABLE 2

Classification of wild chromosomes for viability effects.

ITEM	COLLECTION	NO. TESTS	% *le*+*sle* (SE)	NO. FLIES	% Cy^+ (SE)
1	Mass. 38	151	45.0 (4.1)	40,697	27.42 (0.22)
2	Mass. 41	108	59.3 (4.7)	5,831	26.51 (0.58)
3	1+2	259	51.0 (3.1)	46,528	27.31 (0.21)
4	Fla. 40	227	67.0 (3.1)	5,963	25.14 (0.56)
5	Fla. 42	110	61.8 (4.6)	4,312	25.42 (0.66)
6	4+5	337	65.3 (2.6)	10,275	25.26 (0.43)
7	Ohio	177	49.7 (3.8)	6,819	27.28 (0.54)
8	N. M.	203	62.1 (4.6)	6,225	26.51 (0.56)
9	Maine 38	115	51.3 (4.7)	4,833	28.53 (0.65)
10	Maine 42	111	34.2 (4.5)		

terious in the Fla. area. This difference also has a p value of less than .001. It is clear that so far as the evidence of these two samples goes, the Fla. population is much more saturated with adverse viability genes than is the Mass. population. The proportion of phenotypic visible mutations appears to be the same in both populations (30 per cent of the non-lethal chromosomes).

None of the remaining collections was analyzed genetically in as much detail as the two above collections. All were analyzed sufficiently to show the major characteristics pointed out in the preceding paragraph—namely, the proportion of the chromosomes containing lethal or semi-lethal genes and the mean percentage of non-*Cy* homozygotes in the test generation of all the deleterious and normal lines taken together. These data are given in table 2. Included here are the total number of chromosomes tested for their homozygous effects (No. tests), the percentage of these chromosomes bearing either lethal or semilethal genes (% le+sle), the total number of flies counted in test generations in the deleterious and normal lines (No. flies), and the percentage of non-*Cy* flies in this total (% Cy^+).

The number in the No. flies column is large in the Mass. 38 sample because all lines were carried through several test generations (without a significant change in the percentage of non-*Cy* in the totals for each generation). In the Fla. 40 sample only the lines giving over 28 per cent or between 15 and 20 per cent of

non-*Cy* flies were carried for more than one test generation. In order not to bias the mean percentage of non-*Cy*, only the total number of flies counted in the first test generation were used for this calculation. In the other samples, similar repeated tests were made of lines on the borderline between semi-lethal and deleterious, but only the data from the first test were used in calculating the mean percentage of non-*Cy* for the entire group of deleterious and normal lines.

The major differences between the Mass. 38 and Fla. 40 collections which were pointed out above can be seen by comparing items 1 and 4 of table 2. In the second collections from these same areas, items 2 and 5, these differences are much less pronounced, lacking statistical significance but being in the same direction as in the first collections. In the sums of these data, items 3 and 6, the differences are statistically significant, each p value being less than .001. The Mass. 41 collection differs significantly from the Mass. 38 one with respect to proportion of lethal and semi-lethal chromosomes ($p=.005$); but the differences between the two Fla. samples are not statistically significant. This consideration of items 1 through 6 indicates that in the course of time true fluctuations take place in the genetic content of American *D. melanogaster* populations and that these fluctuations can be great enough to cloud if not obscure differences between widely separated populations. In general, there appears to be a higher saturation of bad viability genes in the Fla. area than in the Mass. one; but it is possible that a maximum has not yet been reached in the Mass. area and that this area will eventually be similar to the Fla. one.

Of the other collections represented in table 2, Ohio and Maine 38 resemble the Mass. collections more than they do the Fla. ones, while N. M. is closer to the Fla. average in percentage of le+sle chromosomes and closer to the Mass. average in the percentage of Cy^+. In the course of three and one-half years in the laboratory there was a significant decrease in the proportion of le+sle chromosomes in the Maine stock ($p=.005$). In both the Maine and N. M. samples the method of collection was such that their data in table 2 represent a much less accurate estimate of the actual characteristics of the natural populations than do the data of the other collections.

The data of table 2 show that in general the proportion of lethal and semi-lethal chromosomes in the populations under consideration averages 50 per cent or more of all the second chromosomes in these populations. In addition, they suggest that there is a higher proportion of bad viability genes in regions where the climate is favorable for breeding throughout the year.

The proportions of non-*Cy* homozygotes in the test generations of all the collections (per cent Cy^+ in table 2) are consistently below the normal expectation of 33.33 per cent. This may mean (1) that nearly all of these chromosomes contain deleterious recessive genes, or (2) that the *Cy* chromosome, after many years of selective laboratory breeding, has accumulated enough favorable dominant viability genes to make it better than a heterozygote of two average wild chromosomes, or (3) that there is a combined effect of these two factors. In order to discover which of these interpretations is correct, the following ex-

periment was carried out. Six average deleterious lines of Mass. 41 were crossed to one or more of 11 average deleterious lines of Ohio immediately after these lines had been isolated from the collections. At the same time, each line was self-mated for an additional test generation. All these matings were made on one day. Their offspring were raised in the same incubator and were counted over the same period of time. The data from these crosses are given in table 3. It can be seen that the proportion of non-*Cy* (28.02 per cent) in the self-matings is not statistically different from the proportion observed in the first test generation in both the Mass. 41 (26.51 per cent) and the Ohio (27.28 per cent) samples and reported in table 2. In the cross matings, however, the proportion of non-*Cy* was much higher (35.03 per cent). The value of p for the difference between these two proportions is well below .0001. It is clear from these data that there is a real difference in the viability of homozygotes in comparison with heterozygotes of representative wild chromosomes when both are raised with heterozygotes of these chromosomes and the *Cy* chromosome. This indi-

TABLE 3

Viability of the Cy L^4 chromosome.

CROSS	*Cy* L^4	+	%+(SE)
Mass. 41×Mass. 41 Ohio×Ohio	1428	556	28.02 (1.01)
Mass. 41×Ohio	2012	1085	35.03 (0.86)

cates that the first interpretation is probably true—that most of these chromosomes do contain genes deleterious to the viability of their homozygotes during development. With regard to the second interpretation, the relative viability of the *Cy* chromosome, the data suggest that the heterozygotes of this chromosome and representative wild chromosomes are actually less viable than the heterozygotes of these same wild chromosomes. The value of p for the difference between the observed proportion of *Cy* in the crossmatings (64.97 per cent) and the expected proportion (66.67 per cent) is .046. It is at least reasonably certain that the *Cy* heterozygote is not more viable than the heterozygote of representative wild chromosomes.

Considering the data of tables 1 and 2 in the light of the data of table 3, it is clear that the large majority of the chromosomes in the deleterious and normal classes contain genes deleterious to their homozygotes during development; and that very few of them, if any, are completely free of such genes.

Mutations affecting the phenotypic appearance of the flies were found in some of the chromosomes in every collection of wild flies. Careful search was made for these in only the Mass. 38 and Fla. 40 analyses, where some of the less evident ones were not noticed until the second test generation. In all of the other collections, however, from 10 to 20 per cent of the non-lethal chromosomes proved to contain genes changing the phenotype noticeably.

Most of the visible mutations were phenotypically weak recessives, many of them overlapping wild type in a large proportion of their homozygotes. Two

weak dominants were found in the Mass. 38 collection, both associated with recessive lethals. Many of the phenotypically strong mutants were either associated with semi-lethals or were themselves semi-lethal. This can be seen in part in table 1, where 50 of 72 semi-lethal chromosomes are listed as showing phenotypic effects, and only 20 of 158 deleterious and normal chromosomes are so listed. There is a clear correlation between visible effect and lowered viability. This suggests that in most of these cases the two effects were produced by the same gene.

Many of the more easily seen mutations proved to be alleles of mutations already recorded in Drosophila literature. These include *net*, *dp*, *b*, *pr*, *cn*, *L*, and *bw* in the second chromosome, *st*, *p*, *gl*, *cd*, and *ca* in the third chromosome, and *sv* in the fourth chromosome. None of these was present in single mutant stocks in the Amherst laboratory at the time of their discovery in chromosomes collected from the wild populations. In addition there were numerous other mutations, many of them apparently at new loci, mostly of little or limited value as chromosome markers. Some of these have been described in various issues of Drosophila Information Service. Others have not yet been studied sufficiently to warrant description.

The finding of third and fourth chromosome mutations is of particular interest because in no case was an exhaustive test made of these two chromosomes. Their discovery indicates that these two chromosomes are probably as much saturated with genetic variability, in proportion to their lengths, as is the second chromosome in the populations under consideration.

Three of the many wild lethal chromosomes reported in these studies are of special interest. One of these, found in Mass. 38, differs from all others observed by the author in that it appears to produce its effect in mid or late pupal development. This results in the production of many dead, black pupae on the sides of the stock cultures. Counts of 1,700 pupa cases by a student, W. D. KEHNE, under the author's supervision, showed that one-third of all the imagos of the *Cy/le* stock die in the pupal period. In most cases the external organs appear to be normal at the time of death, excepting for heavy black pigmentation in the wings and legs. This black condition may be due to post-mortem melanism. No histological analysis has been attempted. This mutation is a true imaginal lethal, in contrast to the others observed, which are either embryonic or larval with respect to the time of lethal effect.

The second lethal chromosome of interest was found in the Ohio collection. Extensive tests through several months after its isolation showed its effect to be such that all females heterozygous for it were sterile, and many of them were weakly Minute in phenotype. It was necessary to carry the chromosome through the males, which were normal in both fertility and phenotype. Two years later it was noted that only an occasional female was Minute, and many tests showed that most of the females were fertile and only a few were completely sterile. No homozygotes appeared, indicating that the nature of the effect of the chromosome had changed to that of a more or less normal recessive lethal.

The third lethal chromosome of interest was another from the Mass. 38 col-

lection. The stock of this lethal regularly gives less than 2 per cent of non-*Cy* flies. These have roughish eyes and an abdomen with broken and missing sclerites. When this lethal chromosome was tested for locus with 2-ple it was found that these two phenotypic effects were caused by separate genes, and that the lethal effect was the result of their combined action. One of the two genes gave 16 per cent of homozygotes in a *Cy*-by-*Cy* mating; the other gave 25 per cent of homozygotes in this type of mating. This is the only instance in this study in which the lethal effect of a wild lethal chromosome has proved to be the result of the combined action of two or more non-lethal genes.

A brief study was made of the distribution of lethal genes in the wild lethal chromosomes. This was done by the tests with the multiple mutant stock, *dp b pr c px sp* (2-ple), which were designed to show which arm of each lethal chromosome carried a lethal gene. A total of 56 wild lethal chromosomes from Maine 38 and Mass. 38 which contained no identical lethal genes, along with 20 lethal chromosomes of independent laboratory origin, was so tested. One of the 56 wild lethal chromosomes was the two-gene lethal referred to above. In the remaining 55 cases, 25 chromosomes had a lethal gene in the left arm (to the left of *pr*), 25 had a lethal gene in the right arm (to the right of *pr*), and the other five had a lethal gene in each arm. In the case of the laboratory lethals, which occurred in chromosomes known to have been previously free of lethal genes, 11 were in the left arm and nine were in the right arm. The total distribution of 41 lethal genes in the left arm and 39 in the right suggests a chance distribution of these lethals through the second chromosome. The finding of more than one lethal gene in some of the wild lethal chromosomes is according to expectation, in view of the high proportion of wild chromosomes carrying lethal genes. It may be added in this respect that in the course of cross-testing the wild lethal chromosomes of each sample, several additional instances were found in which more than one lethal gene was present in a given wild lethal chromosome. Two cases have been found of at least three separate lethal genes in one wild lethal chromosome.

The tests with 2-ple also revealed the presence of any inversions which were present in the lethal chromosomes. Five inversions, three in the left arm and two in the right, were found in tests of 69 chromosomes of wild population origin. Of these chromosomes, 56, in which were four inversions, were found to be lethal at the time of isolation. The other 13 chromosomes, one of which contained an inversion, were non-lethal at the time of isolation from the wild population but had each acquired a lethal through mutation before the inversion test was made. In each case inversion and lethal effect were in the same arm of the chromosome; but in no cases were flies which were made heterozygous for any two of the inversions either lethal or abnormal in appearance.

Inversion tests were also made on 835 chromosomes which were of laboratory origin and were free of inversions in the generation before the test was made. Seven of these chromosomes each represented a laboratory lethal stock. The other 828 chromosomes were from the Canton-Special stock. They were not tested for lethals but could not have contained more than a small proportion

of lethal chromosomes. These Canton tests were made by a student, H. H. SKINNER, under the author's supervision. None of these 835 laboratory stock chromosomes contained an inversion.

The above data represent only the beginning of a study on the rate of spontaneous occurrence of inversions, the proportion of the local wild population chromosomes which carries inversions, and the relation of inversions to lethal genes in American *D. melanogaster*. The only conclusions which can be drawn from them at present are that there is clearly a higher proportion of inversions in the Mass. wild population lethal chromosomes than is found in the chromosomes of an inbred laboratory stock, and that there is a high enough frequency of inversions in the chromosomes of the Mass. population to make further study both possible and worth while.

POPULATION CONTINUITY

One of the third chromosome visible mutations is of particular interest because of the information it gives concerning the continuity of the population in which it was found. This is cardinal (*cd*), a bright red eye color. It was found first in a stock collected in September, 1931, in an area only a few hundred feet from the site of the Mass. 38 collection. It was found again in stocks collected in the same area in the summers of 1932, 1934, and 1938. It appeared in two of the lethal stocks of the Mass. 38 autumn collection. In most of these cases it did not appear in the stock until several generations after the time of collection. It is not surprising therefore that it was not observed in the 1941 collection from this area, since practically all of the chromosome lines of this collection were discarded after two test generation matings. In addition, visible mutations were not looked for as thoroughly in this case as in the others.

No alleles of *cd* were found in any other stocks or collections made by the author during this period. This includes stocks from the neighboring communities of Amherst, N. Amherst, Pelham, and Whately in western Mass., as well as the several collections already discussed in this report. It should be noted that such mutations as *cn* and *st*, both phenotypically similar to *cd* and one of them (*st*) in the same linkage group, were found in one or more of these stocks or collections. Accordingly, the presence of *cd* in this one area over a period of not less than eight years is striking evidence of continuity in this population for at least that length of time.

POPULATION SIZE

The genetic variability found in a population can be used as a yardstick to measure the relative size of the population. In order to do this, certain characteristics of the variability must be ascertained. If the breeding population is large, for instance, then there will be practically no mating between sibs. This will result in a minimum amount of elimination of adverse recessive genes by homozygosis. This in turn will allow a gradual accumulation of such genes in the chromosomes of the population. The rate and amount of accumulation will depend upon a number of factors. Among these are the lethal mutation

rate, the rate at which lethal mutations recur, the number of genes mutating to lethals, the amount of (dominant) selection in heterozygotes against normally recessive lethal genes, and the length of time during which the population is continuously large.

It has already been shown that a large amount of adverse genetic variability is present in the population areas under discussion. Data will now be presented showing the proportion of chromosomes with identical lethal genes. This proportion may be determined for any sample of lethals by crossing the different *le/Cy* lines to each other. If two lines carry an identical lethal, all the offspring of their crosstest will be *Cy*; but if they carry different lethals then up to one-third of their joint offspring will be non-*Cy* heterozygotes of the two lethal genes. By crosstesting only lines giving less than 2 per cent of non-*Cy*, one can easily distinguish between identical lethals and different lethals.

Identical lethal genes may appear in a sample of lethal chromosomes from either one of two causes: (1) independent origin, through recurring mutation of a given gene to a similar lethal form, and (2) common origin, through common parentage of the lethal chromosomes. While it is not possible to determine the nature of the origin of a given pair of identical lethal genes found in a sample of wild chromosomes, it is possible to determine the rate of recurrent mutation. By subtracting this value from the total proportion of identical lethal genes, one arrives at an estimate of the amount of common parentage (inbreeding) represented in the total proportion. In any instance in which all of the total proportion of identical lethals can be accounted for by recurrent mutation, it may be assumed that practically no inbreeding occurred.

The rate of recurrent mutation was estimated by crosstesting lethals which arose independently of each other in the laboratory in chromosomes known to have been previously free of lethal genes. Some of these chromosomes were from the Maine, Mass., and Fla. collections; others were from laboratory stocks of long standing. Some of the mutations occurred in normal environmental conditions, while others occurred in chromosomes which were heat-shocked to an extent only a little more extreme than the shock provided by a hot summer day in any of the areas in which flies have been collected.

Tables 4, 5, and 6 contain the summarized data from the crosstesting of lethal chromosomes.

Table 4 shows the proportion of the crosstests that involved two chromosomes carrying identical lethal genes. The column, le chrom's, shows the number of lethal chromosomes which were crosstested. Where not all of the possible crosstests were completed, this number is enclosed in parentheses. The column, crosstests, gives the actual number of crosstests which were completed. The column, identical, tells how many of these crosstests involved two chromosomes carrying an identical lethal gene. The last column shows the percentage of the crosstests which involved identical lethal genes, and includes the SE of the percentage.

Items 1, 2, and 3 of table 4 are the data from crosstesting two groups of lethals occurring in the laboratory in previously lethal-free chromosomes. The first group in item 1 was discarded before the second group in item 2 was avail-

able. Item 3 is a summation of both groups. It can be seen that seven of 3,031 crosstests involved identical lethal genes, and that the proportion of identical lethals in these samples was .231 per cent. This figure represents the proportion of identical lethals which is to be expected as a result of recurrent lethal mutations. It is the "control" percentage with which the other items of this table will be compared.

An inspection of the percentages of identical lethals in the collections of wild lethal chromosomes represented in the remaining items of table 4 shows at once that these fall into two classes—those which are only a little larger than the control percentage and those which are more than 25 times as large as the control percentage. In the first group are all the cases in which the collection

TABLE 4

Proportion of identical lethals.

ITEM	ORIGIN	*le* CHROM'S	CROSS TESTS	IDENTICAL	% (SE)
1	Lab 1	27	351	0	—
2	Lab 2	(75)	2680	7	.261 (.098)
3	1+2	—	3031	7	.231 (.087)
4	Mass. 38	49	1176	5	.425 (.190)
5	Fla. 40	(50)	1200	4	.333 (.166)
6	Fla. 42	47	1081	6	.555 (.226)
7	5+6	—	2281	10	.438 (.138)
8	Ohio	48	1128	7	.621 (.234)
9	N. M.	48	1128	74	6.56 (0.74)
10	Maine 38	41	820	83	10.12 (1.05)
11	Maine 42	34	561	94	16.76 (1.58)

was made directly in the wild population. In the second group are the samples which were taken from stock bottles and in which it was expected that inbreeding was a dominant factor. Inbreeding was more pronounced in Maine 38 than in N. M. ($p=.003$). It was also stronger in Maine 42 than in Maine 38 ($p=.0004$), showing that a significant change had taken place in this respect during the three and one-half years in which this stock was kept in laboratory culture.

In the Mass., Fla., and Ohio collections the percentage of identical lethals is in each case higher than the controls, but in no case is the value of p as low as .05. It is obvious at once that these percentages much more closely resemble the control percentage than they do the much higher N. M. and Maine percentages. The summation of the Fla. collections in item 7 gives a percentage of identical lethals very close to that found in the Mass. 38 sample. Ohio is highest of this group, though not statistically different from the others.

The data of table 5 show the same facts as the data of table 4, but in a way which sheds additional light upon their origin and meaning. This table shows the number of times that each lethal was observed in each of the collections, this being shown in the columns under Frequency of Appearance. The first three columns of this table are the same as in table 4. The fourth column shows

the number of different lethal genes revealed in these chromosomes through the crosstests. It is the sum of the numbers in the remaining columns of the table.

Items 1 and 2 of table 5 show that in the one case no lethals were found more than once in a sample of 27 lethal chromosomes, while in the second group of 75 lethal chromosomes there were 68 different lethal genes, the other seven chromosomes containing each a lethal that was an allele of one or another of the 68. No lethal genes were found more than twice in this sample. While these data are too few to indicate small differences in mutation rates in genes giving rise to lethals, it seems probable that in the remaining items of this table any instance in which a lethal is found more than three times in a given

TABLE 5

Frequency of different lethal genes.

ITEM	ORIGIN	*le* CHROM'S	*le* GENES	FREQUENCY OF APPEARANCE							
				1	2	3	4	5	6	7	>7
1	Lab 1	27	27	27	0	0	0	0	0	0	0
2	Lab 2	(75)	68	61	7	0	0	0	0	0	0
3	Mass. 38	49	46	43	2	1	0	0	0	0	0
4	Fla. 40	(50)	46	42	4	0	0	0	0	0	0
5	Fla. 42	47	41	35	6	0	0	0	0	0	0
6	Ohio	48	44	42	1	0	1	0	0	0	0
7	N. M.	48	19	7	3	4	3	0	1	1	0
8	Maine 38	41	10	2	1	1	1	1	3	1	0
9	Maine 42	34	17	13	1	0	1	1	0	0	1*

* Appeared 13 times.

sample of lethal chromosomes is much more likely to be a case of common parentage of the chromosomes concerned than of recurrent mutation.

The remaining items of table 5 show that the Mass. and Fla. samples closely resemble the control, while the others differ from it. A total of 12 lethal genes were found twice in these three samples considered together. One was found three times; but none was found more than three times. In the Ohio sample, however, one lethal gene appeared four times in the 48 lethal chromosomes; while only one was found twice and none three times. Recalling the nature of this collection, it seems not unlikely that it represented a mixture of a few flies raised from common parents in the fruit storage and a large number of immigrant flies from outside the storage. The quadruplicate lethal may represent the flies raised in the storage; the others, the immigrant flies. In the N. M. and Maine 38 samples it is at once obvious that a comparatively small number of flies were the parents of most of the flies which were tested. This is particularly true of the Maine 38 collection, where only two lethal chromosomes in a sample of 41 were not duplicates of one or more of the other chromosomes of the sample. In the three and one-half years under laboratory conditions the genetic structure of this culture population changed to a much greater degree than is

suggested by the simple differences shown in tables 2 and 4. As may be seen in this table, the combined forces of chance variation in frequency from generation to generation and of elimination by homozygosis have served to eliminate many of the duplicated lethals while preserving one in a large proportion of the chromosomes (13 of 34 tested ones). In addition it appears probable that most of the 13 lethals found only once in these 31 chromosomes represent new lethals which have occurred in the laboratory and have accumulated during the many generations following the collection in the Maine area.

Taken together, the data of tables 4 and 5 suggest that at least in the Mass. and Fla. areas recurrent mutation, rather than inbreeding, is the cause of the appearance of identical lethals in the wild chromosomes collected in these areas.

In order to compare more closely the proportions of identical lethals in the

TABLE 6

Corrected proportion of identical lethals.

ITEM	ORIGIN	CALC. TESTS	IDENTICAL	% (SE)
1	Controls	3031	7	.231 (.087)
2	Mass. 38	2043	5	.244 (.109)
3	Fla. 40	3253	4	.123 (.061)
4	Fla. 42	2559	6	.234 (.095)
5	Total	10886	22	.202 (.043)

samples of Mass. and Fla. wild lethal chromosomes with the proportion of identical lethals in the sample of lethals occurring in the laboratory, it is necessary to take into account the actual number of lethal genes which the wild lethal chromosomes carry. In view of the data already presented, the simplest assumption is that lethals accumulate in the chromosomes in a Poisson series, resulting in a Poisson distribution of chromosomes carrying respectively two, three, four, etc., lethals per chromosome. This calculation has been carried out, with the aid of PROFESSOR G. P. CHILD, in a manner identical with the similar calculation by DOBZHANSKY and WRIGHT (1941). Using the data of this calculation, it is possible to estimate the average number of lethal genes carried by each wild lethal chromosome, and from this to estimate the most probable number of crosstests made for each of the three samples of wild lethals. The calculated number of crosstests has been corrected in addition for the crosstests which could not be made—namely, those involving lethals carried in the same chromosome. The corrected calculated number of crosstests forms the basis for a restatement of the pertinent data of table 4 in table 6. It may be seen at once that this correction brings all three percentages of identical lethals into the immediate range of the control percentage. Indeed, the value of p for the distribution of these four proportions around their mean is .70, indicating the high probability that there is no difference between them. The conclusion to be drawn from this table of corrected data is that all of the identical lethal

genes found in these collections may be satisfactorily explained as cases of recurrent mutations. The amount of common parentage in the lethal chromosomes in these three collections of wild chromosomes appears negligible.

It is possible to estimate the minimum number of genes in the second chromosome in *D. melanogaster* which mutate to lethal alleles by using the data of table 6. The average proportion of identical lethals in the four samples of lethal chromosomes comprising this table is .00202. This means that an average of one in every 495 crosstests of lethal chromosomes which contain a single lethal mutation will involve two lethals which are alleles, and that there are 495 ($\pm$ 106) genes mutating to lethals in this chromosome. This conclusion rests upon the assumption that all these genes mutate to lethals at approximately an equal rate. While this is probably not strictly true, there is no evidence to the contrary in the data upon which the estimate is based.

The next factor to be considered in this section is the lethal mutation rate per chromosome per generation under various environmental and genetic conditions. Many data exist showing that the mutation rate responds to a number of environmental and genetic agents. In the present instance interest has centered around the mutation rate in stocks newly isolated from the wild population, in order to secure an estimate of the mutation rate in the wild population itself. No attempt has been made to duplicate the experiments of ZUJTIN and PAVLOVETZ (1940) and ZUJTIN (1940a, 1940b). Data have been collected on the mutation rate in lines heterozygous for *Cy* and the non-lethal chromosomes of the Mass. 38 and Maine 38 collections, in ten lines each homozygous for one of the deleterious chromosomes of the Fla. 42 collection, and in the Canton-Special stock perfected by BRIDGES. In the Fla. 42 and Canton tests data were collected separately for male and female lines in most of the series. In all cases some series were raised at constant temperatures ranging between 22 and 28°C, while others were heat-shocked in the generation in which the mutations occurred.

The scheme of matings used in these tests was essentially the same as has already been described for isolating lethal wild chromosomes. In the series beginning with a fly from a $+/Cy$ line a test mating was made of its $+/Cy$ offspring in order to eliminate cases in which the parent fly carried a lethal which had occurred in the stock since its isolation. In the series beginning with a fly from a line that was originally $+/+$, it was necessary to test many of the chromosomes from each fly in order to eliminate from consideration those which chanced similarly to be $+/$le. Not fewer than 12 chromosomes, and usually 20 or more, were tested from each wild type fly.

PLOUGH collected the data of the Maine 38 series. Students, W. G. METCALF and J. J. FOSTER, did the experiments with the Canton stock, under the joint supervision of PROFESSOR PLOUGH and the author; students, J. E. EDWARDS and H. G. STORRS, were technical assistants to the author in the Fla. 42 series.

The data from these mutation rate experiments appear in table 7. The data from the series raised at 22 to 28°C appear in the columns under Control; those from series exposed during larval or pupal development to 36.5°C for 12

to 24 hours appear in the columns under Shocked. The sums of the two groups of data appear in each case in the columns under Total. In each group of data are the number of chromosomes tested (tests), the number of lethal chromosomes found (le), and the percentage of lethal chromosomes (% le). Items 3, 4, and 5 have appeared in PLOUGH (1941). The other items represent data not previously published in more than abstract form. The data of the Fla. 10 sel. stock, are from previous experiments by PLOUGH and CHILD. The stock was a highly inbred laboratory one coming originally from Florida. In their experiments they tested the mutation rate in $+/Cy$ flies. Item 2 is the summed data of eight of the ten Fla. 42 lines, the remaining lines appearing in items 6 and 7, respectively.

Considering first the effects of different genotypes on the mutation rate, it is

TABLE 7

Lethal mutation rates in different stocks.

ITEM	ORIGIN	CONTROL			SHOCKED			TOTAL		
		TESTS	*le*	% *le*	TESTS	*le*	% *le*	TESTS	*le*	% *le* (SE)
1	Canton 41	1022	5	0.49	1897	10	0.53	2919	15	0.51 (0.13)
2	F. 42 Low	672	4	0.60	815	9	1.10	1487	13	0.87 (0.24)
3	F. 10 sel.	979	8	0.82	452	6	1.33	1431	14	0.98 (0.26)
4	Maine 38	189	2	1.06	348	6	1.72	537	8	1.49 (0.52)
5	Mass. 38	754	13	1.74	500	5	1.00	1254	18	1.44 (0.34)
6	F. 42 Med.	132	4	3.03	103	4	3.89	235	8	3.40 (1.18)
7	F. 42 Hi.	129	8	6.20	220	17	7.73	349	25	7.16 (1.38)
8	2+3	1651	12	0.73	1268	15	1.18	2918	27	0.93 (0.18)
9	4+5	943	15	1.59	848	11	1.30	1791	26	1.45 (0.28)

apparent at once in the data of table 7 that a large variation exists in the mutation rates of these different stocks and lines. The most obvious difference is between the "low" lines of Fla. 42 in item 2 and the two "higher" lines, "medium" in item 6, and "high" in item 7. Details of the analysis of these lines, along with further data on the high line, will appear in a separate publication. It may be said here that the most probable interpretation of these data is that there were three different mutation rates represented in these ten lines; and that the higher rates in items 6 and 7 were caused by one or more mutations present in the respective second chromosomes for which these lines are homozygous. These tests were made immediately after the lines were isolated from the wild collection. Eight months later a new series of tests was run on the high line and on one of the low lines to determine the sex-linked lethal mutation rate. In those tests the high line of item 7 was as much above the low line as in these data on second chromosome lethals. This indicates that the effect is general on all the chromosomes. In addition, the data from the crosstests of the second chromosome lethals indicated clearly that the high mutation rate was not produced by a specific increase in mutation rate in gonial cells resulting in

many duplicate lethal germ cells; while the data on the sex-linked lethals suggested that the effects of the high mutation rate factor may be considerably increased with the age of the fly, possibly above what has been observed by RAJEWSKY and TIMOFEEFF-RESSOVSKY (1939) in lines showing a normal low mutation rate.

The data of items 4 and 5 in table 7 for the most part do not represent sufficient tests of any one line of the stocks concerned to indicate the presence of dominant genetic heterogeneity influencing the mutation rate. Thus, the 537 tests of Maine 38 represent fairly evenly 56 parent lines of $+/Cy$. In the Mass. 38 data 764 tests (12 lethals) represent 86 parent lines; while the remaining 481 tests (five lethals) represent four lines. In this last case the distribution of the five lethals in the four lines has a p value of .17, showing no evidence of difference in mutation rate in these four lines.

The data of items 1 through 5 of table 7 suggest that differences exist in the mutation rates of these stocks; but the data are not large enough to make these differences statistically significant in most of the individual comparisons. Taking them as a whole, however, they suggest differences between Canton and the two Fla. stocks, between Canton and the two New England stocks, and between the two Fla. stocks and the two New England stocks. There are no consistent or strong differences between either the two Fla. stocks or the two New England stocks, leaving out of consideration items 6 and 7. If one compares the two geographical areas and Canton by adding the two Fla. stocks and the two New England stocks together then the differences between the three resultant groups of data, items 1, 8, and 9 of table 7, reach statistical significance. For the controls the p value for the difference between the three mutation rates is .02; for the shocked series, .05; and for the totals it is less than .01. It appears likely that the differences between the geographical areas in mutation rate, and in turn between each of them and the Canton laboratory stock, are manifestations of genetic differences in mutation rate not unlike those found by DEMEREC (1937) between stocks collected in different areas.

The environmental factor, temperature shock, appears to have had very little effect on the lethal mutation rate in the stocks of table 7. There is a suggestion of an increase in both the Fla. 10 sel. stock and the Fla. 42 low lines; but even in the combined data of item 8 the total numbers upon which the difference in percentile rate is based are so small that the value of p for the difference between control and shocked series is only .20. The actual increase in rate is sufficient, however, to eliminate the difference between the Fla. stocks and the New England ones in the shocked series. The data of item 2 thus are in line with PLOUGH's hypothesis (1941) that temperature shock raises the rate in low lines to the level of the higher mutation rate stocks. The data of the Canton stock, however, show no such effect. This may mean that some low mutation rate stocks do not respond to temperature shock with an increase in mutation rate, and that this is another type of genetic difference between stocks.

The lack of difference between the mutation rates in males and females in these experiments is shown by the data of table 8. In this table are six inde-

pendent samples of data from males and females which were in each case members of the same stock or line and thus were genetically similar with respect to mutation rate genes. The first item contains data from the series involving the Canton stock. The other samples are from the Fla. 42 lines. Item 2 is the control of the low lines; item 3, the shocked series of the low lines. Item 4 is the Fla. 42 medium line. Item 5 is the X chromosome data of young females and young males of the Fla. 42 high line; and item 6 is the second chromosome data of this stock. In items 1 and 4 the male lines have the higher rate; in the other items the female mutation rate is the higher. In no single instance is there a statistical significance in the difference between the sexes with respect to the mutation rate. The value of p for a group of six such distributions, given an expectation of no difference between the two sexes, is .48, indicating a close fit to expectation. There is nothing in the data of these five thousand tests that suggests a difference in the mutation rate, particularly the autosomal mutation rate, in the two sexes within a given homozygous stock of flies.

TABLE 8

Mutation rates in females vs. males.

ITEM	SERIES	♀ TESTS	*le*	% *le*	♂ TESTS	*le*	% *le*
1	Canton	813	2	.25	1478	11	.74
2	Fla. LC	345	3	.87	327	1	.31
3	Fla. LS	254	3	1.18	561	6	1.07
4	Fla. Med.	111	3	2.70	124	5	4.03
5	Fla. HX	413	5	1.21	244	2	.82
6	Fla. HII	174	16	9.20	175	9	5.14
	Totals	2110	32		2909	34	

When the proportion of lethal genes in a wild population has reached a stationary level, there exists an equilibrium between the introduction of new lethal genes in each generation by process of mutation and the loss of lethal genes in each generation through the effects of these genes on the individuals which carry them. The most obvious type of loss is elimination by homozygosis. In no studies thus far reported, however, has this one source of loss been sufficient to balance the mutation rate, even in instances where there was reason to believe that the proportion of lethal genes has probably reached its peak. This has led to the hypothesis that the remainder of the loss is due to elimination by dominant selection against these recessive lethals. That such a situation exists in the present study is indicated by the data of table 9, which shows the estimated amount of elimination by homozygosis in the Mass. and Fla. populations.

The data of table 9 include the observed proportion of wild chromosomes carrying lethal genes (% le), the proportion of the crosstests of these chromosomes which involved identical lethal genes (% same), the average number of lethal genes per lethal chromosome (genes), and finally the estimated

amount of homozygosis occurring in the total matings in the population in the next generation (% out). This last figure is the rate of elimination by homozygosis. Its calculation is based upon a method introduced by STURTEVANT (DOBZHANSKY 1939). It is the square of the proportion of wild chromosomes carrying lethals, multiplied by the proportion of identical lethal genes, this product in turn being multiplied by the average number of lethal genes per lethal chromosome.

The elimination rate of lethal genes (% out) in table 9 is stated in the same terms as the mutation rate (% le) in table 7, thus making possible a direct comparison between these two rates in each of the two population areas. It may be seen that the mutation rates (items 5 and 2, Total, table 7) are much

TABLE 9

Elimination of lethals by homozygosis.

ITEM	ORIGIN	% *le*	% SAME	NO. GENES	% OUT
1	Mass. 38	45.0	.425	1.33	.115
2	Fla. 40	67.0	.333	1.66	.249
3	Fla. 42	61.8	.555	1.56	.331
4	2+3	65.3	.438	1.61	.301

higher than the elimination rates of table 9 in each instance. The Fla. 42 mutation rate is three and one-half times the Fla. 40 elimination rate and two and one-half times the Fla. 42 elimination rate. The Mass. mutation rate is twelve and one-half times the Mass. elimination rate. The data of the Mass. 41 collection (table 2) show a significant increase in the proportion of lethal chromosomes in the population during the three-year period between collections. This may result from the difference between the mutation rate and the elimination rate. In the case of the Fla. area, however, there was no significant change in the two years between collections; and some hypothesis, such as dominant selection against lethals, is necessary to account for the lack of equilibrium between mutation and elimination rates.

The average amount of dominant selection against lethal genes necessary to produce equilibrium between mutation and elimination rates in the Fla. population is too small to measure experimentally. It amounts to only .57 per cent per generation. It is possible, however, that under certain environmental conditions dominant selection against lethals might be considerably greater than average. If, for instance, a small but random sample of lethals were selected against to the extent of 50 or 25 or even 10 per cent in each generation, this could easily result in such a small average elimination in the population as the .57 per cent rate above. Selection rates of this much larger order of magnitude can be easily measured experimentally.

Just such an experiment has been carried out by the author and three of the students mentioned above, FOSTER, METCALF, and STORRS. In our work with the Canton stock we were testing the mutation rate at constant temperatures

of 14, 19.5, 25, and 30.5°C; and in lines raised at 25° but shocked either during the late larval or late pupal periods with 12 to 24 hours of 36.5°C. Inasmuch as we were testing the mutation rate in wild type flies, it was necessary to test a dozen or more germ cells from each fly to eliminate those families coming from a fly which happened to be +/*le* at the start as the result of an earlier lethal mutation. As an aside to our mutation experiment, records were kept on the numbers of lethal and non-lethal germ cells recovered from flies which were +/*le* at the start, to see if the expected 1:1 ratio was realized. These lethals were cultured for a second generation and tests were made in each family to make sure the lethals were all duplicates. When the data of these several temperature series were completed, it was obvious at once that the 1:1 ratio was realized in all but one of the series—the pupal shock series. MR. FOSTER was responsible for the data in this series. He made counts in all the lethal and non-lethal lines in each of his lethal families. There were no lines in which dis-

TABLE 10

Effect of temperature shock on lethal germ cells.

SERIES	GERM CELLS RECOVERED		
	+	LETHAL	% *le* (SE)
Controls	186	183	49.6 (2.6)
Shocked ♀	110	51	31.7 (3.7)
Shocked ♂	94	61	39.4 (3.9)
Total shocked	204	112	35.4 (2.7)

tinction was difficult or doubtful. He had in addition kept separate records for male and female families. The combined data of these different temperature series appear in table 10. The controls include the data from all the lines excepting the pupal shock ones. It may be seen that in these families from +/*le* flies 186+ germ cells and 183 lethal germ cells were recovered in the next generation. But in the pupal shock series, in both male and female families, there was a deficiency amounting to nearly 50 per cent in the number of recovered lethal germ cells. The value of p for the difference between shocked and controls is .0003. These data involve three lethal genes, in unknown proportions. It is obvious that more data must be collected on a larger sample of lethals before the fact of dominant selection can be accepted as proven. But as far as they go, these data support the modified concept of dominant selection under special conditions outlined above. There may well be other environmental agents which affect +/+ and +/*le* individuals differentially at one stage or another in their development, and which do so in natural populations.

No attempt has been made to observe directly for how long a period of time the breeding populations of *D. melanogaster* have been continuously large in any of the areas reported in this study. This would involve the analysis of at least annual collections from a given area for a period of many years. By the

combined use of certain of the preceding data, however, it is possible to estimate the approximate amount of time this condition of a continuously large breeding population would have to be maintained to reach the observed level of saturation of lethals in the chromosomes. In order to do this, certain assumptions must be made. Probably the simplest situation is described by assuming that in the beginning a certain level of lethal mutations existed in the population, that the observed level is the result of a continuous and constant accumulation of new lethal mutations in the nature of a Poisson series in the chromosomes during the intervening time, and that the observed proportion of lethal genes per chromosome represents an equilibrium proportion in which gain by mutation is balanced by loss through elimination—both by homozygosis and by dominant adverse selection.

The only area in which such a calculation has been attempted is that of the Mass. collections. In this area the data on *cd* indicate a continuous population; but environmental conditions are certainly not so favorable for the continuation of as large a population as in Fla. The lethal mutation rate for chromosomes from this area has been observed to be .0144 lethal genes per chromosome per generation. The calculated proportion of lethal genes in the chromosomes of the wild population, based on a Poisson accumulation of lethal genes in the lethal chromosomes, is .598 lethal genes per chromosome. The observed elimination rate by homozygosis was .0011 lethal genes per chromosome per generation. If, however, in the beginning there was a low proportion of lethals, the loss by homozygosis would be negligible. The time for increase in proportion of lethals to a given level then will depend largely on the amount of dominant selection against lethals. The minimum time necessary for such an accumulation will be found by assuming that under certain conditions there is no effective dominant selection against recessive lethals. If, under such optimum conditions of no elimination by either homozygosis or dominant selection, the lethal level were to rise from that observed in most of the Russian populations (roughly .100 lethals per chromosome) to the level observed in the Mass. 1938 population, a period of 35 generations, or about five years, would elapse. On the other hand, if there were as much dominant selection throughout the period of accumulation as would have to exist to keep the lethal level at the 1938 point, then there would be a net addition of only .0013 lethals per chromosome per generation (still assuming no loss by homozygosis). The duration of the accumulation would then amount to 383 generations, or about 55 years. The data of the Mass. 1941 collection indicate that the proportion of lethals increased in the three years between collections, showing that equilibrium had not been reached in 1938 and that the mutation rate was still in excess of the elimination rate. This suggests that dominant selection against lethal genes is probably not at the maximum value. Thus the best estimate seems to be that the period of accumulation in the Mass. area lies somewhere between five years at one extreme and 55 years at the other. In this respect it is interesting to note that large commercial apple production in this area dates from 30 years before the 1938 collection, a point of time midway between the above

estimated extremes. It is therefore possible that a direct relationship exists between the orchards and the assumption of the characteristics of a large breeding unit by the local *D. melanogaster* population.

DISCUSSION

From the foregoing data certain conclusions have been drawn concerning the structure of natural populations of *Drosophila melanogaster* in the major part of its range in at least the United States of America. While these conclusions are based for the most part on data from collections in Massachusetts and Florida the data of collections in Maine, Ohio, and New Mexico, though less extensive and less complete, are in accord with these general conclusions. It is not unreasonable to suppose that population characteristics correlated with the Massachusetts and Florida data obtain in general throughout the entire range of the species in North America. The two major conclusions are, first, that American *Drosophila melanogaster* breeds in comparatively large populations; and, second, that these large populations are continuous from year to year not only in tropical and subtropical areas but also in the temperate zones of the northern United States. These conclusions are based on three lines of evidence: the low proportion of identical lethal genes found among lethal chromosomes in Massachusetts and Florida collections; the high proportion of lethal chromosomes in these collections; and the continuous presence of the infrequent mutation, cardinal eye color, in collections from one Massachusetts area over a period of eight years. The first of these lines of evidence indicates a large breeding population continuous for at least several generations, since the proportion of identical lethal genes among wild lethal genes was no greater than that among a sample of lethals which arose in the laboratory independently of each other. The second, considered in conjunction with the first, suggests an accumulation of lethal genes over a long period of time. The third indicates a population continuous for at least eight years in the area in which the collections were made.

The conclusion that the breeding populations of *Drosophila melanogaster* in America are continuously large is of particular interest because this is a condition not found in Russian *Drosophila melanogaster* populations and in American populations of several other Drosophila species, including *pseudoobscura*, *hydei*, *immigrans*, and *virilis*. In all of these other cases the reported data are such as to lead to the conclusion that at least at the time when the breeding population is at its smallest number in the annual cycle of numerical size it is split up into comparatively small breeding units. One possible exception to this generalization is *D. hydei* which in Southern California appears to breed in continuously large populations in fruit dumps (SPENCER 1941). In Russian fruit orchard populations the proportion of lethal chromosomes was generally less than one-third as large as the proportions in the Massachusetts orchard area, while the proportion of identical lethal genes in the Russian collections was ten times as high as in the Mass. 38 collection. The same general difference in proportion of lethal chromosomes is apparent between all the Russian collections as a group

and all the American collections as a group. There can be little doubt that these are real differences. Presumably they arise from differences in the structure of the breeding populations of this species in the two continents, particularly differences in breeding population size. Why these differences should exist to such a marked extent is not at all clear.

The differences between American *D. melanogaster* populations and the populations of the other studied species of American Drosophila noted above may trace back to differences in ecological habits. *D. pseudoobscura*, for example, in the desert mountain populations most studied by DOBZHANSKY and coworkers, is generally able to be active for only a small portion of each day. In addition, its populations in this case are sharply discontinuous in distribution. In the area of the Massachusetts population, on the other hand, *D. melanogaster* can be observed to be very active throughout an average summer day; and its range of habitation is practically continuous from there to the regions of the Maine, Ohio, and Florida collections. This is not to say that there is no such thing as a local breeding population in the eastern United States. Indeed, the data on cardinal eye color are evidence that a local population does exist. These considerations do indicate, however, that much larger population units and more mixing between units are to be expected in American *D. melanogaster* than in American *D. pseudoobscura* free-living desert mountain populations.

An additional ecological factor which may be of some importance is suggested by a comparison of the data of the two Florida collections of *D. melanogaster*. These samples differed principally in the manner of their collection. The first collection was derived from a few heavily infested fruit gathered under one tree. The second consisted of adults collected in the same area a year later. It is not unreasonable to suppose that the flies in the first collection would include a much larger proportion of sibs than would the second. This would show up as a higher proportion of identical lethals in the first collection, a proportion of the order of that found in the New Mexico collection. Actually, there was no higher proportion of identical lethals in this collection than in the second Florida collection. In fact, the proportion of identical lethals in this first Florida collection was the lowest found in any of the collections and was lower than the proportion found in laboratory lethals of independent origin, none of these differences, however, being of statistical significance. This fact suggests strongly (but does not prove) that the females of *D. melanogaster*, in this particular area at least, tend to lay very few eggs on each decaying fruit—possibly as few as one egg—and that many females oviposit on each decaying fruit. There is here evidence which suggests that at the time of egg-laying the *D. melanogaster* female seeks out many places to lay her eggs. This could be an important factor in maintaining the characteristic of a large breeding population for this species.

The studies of TIMOFEEFF-RESSOVSKY and TIMOFEEFF-RESSOVSKY (1940a, 1940b, 1940c) and of DOBZHANSKY and WRIGHT (1943) on the distribution and dispersion of *D. melanogaster* led them to conclude that this species may well have the same small breeding unit which characterizes other Drosophila. It may be that the species is more active in its natural habitat in America than

it is in either Berlin or the American habitat of *D. pseudoobscura*. It would be interesting to repeat the "match races" of DOBZHANSKY and WRIGHT on *D. melanogaster* "home grounds" and observe the results. Even if *D. pseudoobscura* were again returned victor, it is difficult to see how this would alter the above interpretations of the other data collected in these studies of the genetic structure of natural populations of Drosophila.

The importance of the difference in size of breeding population in American *D. melanogaster* contrasted with other Drosophila populations is that while the latter possess the evolutionary potentialities of small or medium sized populations, the former possess the evolutionary potentialities of large breeding populations. If this difference in size continues long enough, it must eventually result in different evolutionary consequences in the two kinds of populations. The evolutionary course will be comparatively slow and insecure in the continuously large populations and comparatively fast and secure in the smaller populations. Details of the argument on which this expectation is based and further considerations arising from size differences in breeding populations have already been reviewed and discussed by DOBZHANSKY (1941) and others and need not be treated further here. One major detail worth particular notice is the fact that American *D. melanogaster* chromosomes are now so saturated with adverse viability genes that almost no individual homozygous for any one of the major autosomes has a survvial expectation as great as the average heterozygote. Heterosis in this species, particularly in the sense described by DOBZHANSKY et al. (1942), appears to be the main force insuring its continuation while in competition with other species carrying a much lower amount of homozygous genetic adversity. It is difficult to see how American *D. melanogaster* could long survive competitively in true small breeding populations if conditions suddenly forced it into this state for any considerable period of time.

A question which naturally arises in a discussion of the point of a continuous population is how and where do flies overwinter in regions which are characterized by several consecutive months of sub-freezing temperature? SPENCER (1941) and in a personal communication cites evidence that in Ohio *D. melanogaster* overwinters in farm buildings. Collecting early in the spring, SPENCER repeatedly found individuals of this species in traps nearest to farm buildings and only later in traps near orchards or woods and away from the buildings. In a small and probably inadequate series of attempts in the winter of 1938–1939 the author found no Drosophila in several farm fruit cellars in the Massachusetts area, either as adults or as larvae. Occasional adults of *D. funebris* were found in one farm kitchen and of *D. hydei* in a suburban kitchen. No attempts have been made in the laboratory to reproduce farm building conditions and to test the ability of *D. melanogaster* to survive such a winter. In one accidental case, however, two culture bottles of Canton-Special larvae survived two months at 8°C and proceeded to develop into fertile adults. If it should prove that both adults and larvae can overwinter, then it will be possible to interpret SPENCER'S collection records differently. They might mean that adults survive for the most part only in buildings, but that larvae overwinter in decaying fruit wherever such is kept above freezing and still at a low

enough temperature to delay development of the imago until spring. Completion of the development of larvae overwintering out-of-doors might consume enough time to account for SPENCER's collection data. Whatever may be the answer to this question, there can be no doubt that overwintering does occur and that it occurs in large enough numbers of *D. melanogaster* in the northern United States to qualify it now as a native species of this zone of temperate climate.

From the data on the rate of recurrent lethal mutations both among lethals occurring in the laboratory and among lethals found in wild collections, where such collections did not appear to differ from the laboratory series in their proportions of identical lethals, it has been estimated that 495 ± 105 genes have contributed to the lethal mutations found in these samples. The distribution of detected second and third mutations of these genes is such as to suggest that each of the individual genes of this group mutates at approximately the same rate. The estimate of 495 genes compares favorably with a similarly made estimate by WRIGHT, DOBZHANSKY, and HOVANITZ (1942) of 285 genes mutating to lethals in the third chromosome of *D. pseudoobscura*. This element in *D. pseudoobscura* is genetically very much the same as the right arm of the *D. melanogaster* second chromosome, which represents roughly one-half of the genetic material of the entire second chromosome. It can be seen that twice the *D. pseudoobscura* estimate, 570 genes, falls well within the SE of the *D. melanogaster* estimate.

The weight of these two independent pieces of evidence cannot be ignored in speculative deductions concerning the number and the nature of the genes in Drosophila chromosomes. From their studies on salivary gland chromosomes BRIDGES and BRIDGES (1939) have estimated the number of bands in this particular chromosome material to be not less than four times as large as the above estimates of gene number based on lethal frequencies. It is generally supposed that each salivary chromosome band represents at least one gene. Thus it can be seen that there is a marked difference in the number of genes estimated by these two independent methods. One way of interpreting this difference, taking both estimates as substantially correct, is to assume that genes may differ in the types of mutations which they generally undergo. One class of genes may be of a type which usually gives rise to recessive lethal mutations, either because of their own chemical nature or because of the developmental processes which they and their mutations particularly control. A second class of genes may give rise mainly to phenotypic mutations without the marked effect of viability that characterizes lethal mutations. A third class may be of such a nature that mutations in them are generally dominant lethals. A fourth class may be so stable that they rarely mutate at all. It is not necessary to assume that a given gene always mutates to one type of mutation, but only that as a general rule its most frequent type of mutation is one of these types and not another. Estimates of gene number based on chromosome structure would include all of these classes of genes; while those based on a more limited technique as in the present study, would include only such genes as fall within the detective power of the particular technique employed. At

least it can be seen from these speculations that the different estimates of gene number noted above need not necessarily be considered contradictory.

A good deal of evidence has now been published indicating that the mutation rate differs in laboratory fly stocks collected in different regions of the world and that it is to some extent under the control of mutation rate genes. Several instances have now been reported of spontaneous mutation rates, and changes in them, of such an order of magnitude as to be genetically spectacular. (See especially NEEL 1942 and MAMPELL 1943.) The data from the analysis of ten homozygous wild chromosome lines of the Florida area, together with the data from the other stocks reported in this paper, not only add new instances of these phenomena but also indicate that even in one local area the mutation rate in different flies of the wild population may differ strikingly under certain conditions. This makes difficult an accurate estimate of the average mutation rate, particularly over a long period of time, in a given population area.

The finding of no difference in the mutation rate in the two sexes, with particular regard to second chromosome lethals, is of interest because it supports the findings of PLOUGH and IVES (1935) with regard to autosomal visible mutations and at the same time is in strong contrast with the findings of AUERBACH (1941) in the case of sex-linked lethals. She found a mutation rate approximately nine times as high in sperm as in eggs. The only data on sex-linked lethals in the present study are those in the Florida high line, where the mutation rate was actually, though not significantly, higher in eggs than in spermatozoa. (See table 8, item 5.) The number of tests in this one case is sufficient to indicate a statistical difference from AUERBACH's results. While it is possible that the differences between the data of AUERBACH and these other data are due to differences in experimental conditions, it should be recognized that they may reflect differences in the effects of sex on the mutation rate in the two kinds of chromosomes, sex and autosomal, and that this effect is changed by this particular high mutation rate gene. AUERBACH's work was done with a Florida stock which showed a mutation rate similar to that found in the Fla. 42 low and Fla. 10 sel. lines of the Amherst experiments. Critical evidence should come from a concomitant study of the sex-linked and autosomal lethal mutation rate in sib sperm and eggs in both low and high mutation rate lines.

The lack of significant increase in the second chromosome lethal mutation rate following temperature shock is in contrast with the data of both BUCHMANN and TIMOFEEFF-RESSOVSKY (1936) and PLOUGH and IVES (1935). The former found a two-fold increase in sex-linked lethals, the latter a six-fold increase in autosomal visible mutations, following temperature shock in the late larval stage of development. The present data (table 7) are not sufficient in number to indicate a significant difference from the effects of temperature shock on the X chromosome lethal mutation rate. There is, in fact, in all cases but one in these data, a slight increase in the mutation rate in the shocked series. But the data of this report, of PLOUGH (1941), and of BUCHMANN and TIMOFEEFF-RESSOVSKY (1936) do differ significantly from PLOUGH and IVES (1935), suggesting strongly that there is no such increase in lethal mutations as in at least autosomal visibles following temperature shock. This is the more sig-

nificant when it is recognized that much of the data of the three reports from this laboratory are from identical stocks or from stocks collected at different times in the same geographic area. It seems worth while, therefore, to suggest that there may be a differential effect of temperature shock such that it produces comparatively more visible mutations than lethals. Critical evidence on this point should result from extensive concomitant tests on the lethal and visible mutation rate in control and shocked series of at least one homozygous stock.

SUMMARY

Data are presented showing the proportion of wild second chromosomes of *D. melanogaster* in five areas of the United States carrying mutant genes influencing the viability of the homozygote. This proportion varies from 45 to to 67 per cent of lethal and semi-lethal chromosomes and rises to nearly 100 per cent in some cases when lesser deleterious chromosomes are included.

The incidence of identical lethal genes among samples of lethal chromosomes collected individually in wild populations in Massachusetts and Florida was the same as in samples of lethals occurring independently of each other in the laboratory. This indicates that no significant amount of inbreeding was taking place in these populations at the time the collections were made.

The presence of the mutation cardinal, *cd*, in one Massachusetts area and not in neighboring areas or in other collections, through a period of eight years, indicates a continuous and to some extent individual breeding population in this area.

These data suggest that *D. melanogaster* breeds in large populations in America, in contrast to the smaller populations characteristic of other studied American Drosophila as well as of *D. melanogaster* in western Russia. They also suggest that this species is now a native species of the temperate climatic zone of the United States.

It is suggested that these differences in the nature of their breeding populations, if continued for a long enough period of time, should lead to a slower and less secure evolutionary trend in American *D. melanogaster* than in Drosophila populations of smaller breeding size. Heterosis appears to be a dominant factor in maintaining populations of American *D. melanogaster*.

Studies on the second chromosome lethal mutation rate in several stocks and in ten homozygous lines collected in one Florida area showed (a) no effect of temperature shock on the mutation rate, (b) no difference in the mutation rate in females versus males, and (c) the presence of marked differences in mutation rate both between stocks and between the ten homozygous lines. When compared with the data of other publications, (a) and (b) suggest the existence of differential effects of temperature shock on the mutation rate in the X chromosome versus the autosomes and of lethals versus visibles, as well as differences in the effect of sex upon the mutation rate in X chromosomes versus autosomes. The existing data, however, are not such as to establish these conditions beyond reasonable doubt. In the data of (c) a new case of unusually high spontaneous mutation rate is reported, and it is suggested that even in a local area

the mutation rate is not always inherently the same in all members of the population.

The number of genes mutating to lethals in the second chromosome is estimated to be 495 ± 105. This compares favorably with the previous estimate of 295 genes mutating to lethals in the 50 percent smaller third chromosome of *D. pseudoobscura*, but is less than 25 percent as large as the number of bands visible in salivary gland preparations. It is suggested that this may reflect the presence of different kinds of genes in the chromosomes all of which contribute to the structure of the salivary chromosomes but only part of which mutate to lethals.

Data are presented showing that temperature shock may act as a natural agent of (dominant) selection in heterozygotes against lethals in the gonial or germ cell stage. It is suggested that the small average amount of dominant selection against recessive lethals necessary to maintain equilibrium between mutation and elimination of lethals in the wild population may be effected by a much larger average amount of dominant selection against a small random sample of these lethals in each generation, this selection being the result of adverse environmental conditions.

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THE OCCURRENCE OF X-RAY INDUCED DOMINANT LETHAL MUTATIONS IN HABROBRACON[1]

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INTRODUCTION

THE PARASITIC wasp, Habrobracon, has been considered of special value in connection with studies of dominant lethals because of its somewhat exceptional method of breeding. In "out-crosses," now called three-allele crosses because they involve triple alleles at the sex locus, all fertilized eggs develop into females, unfertilized ones into males (WHITING 1937a, 1940, 1943). It follows, then, that if a sperm bearing one or more dominant lethals fertilizes an egg, a potential female is lost. Statistically the result of an appreciable number of such events would be a depression in the number of females. But the number of males should remain unaltered, since the frequency with which they occur is determined by the proportion of cases in which sperm fail to enter the eggs, and not, therefore, by the constitution of the sperm. Sex-ratios, then, would be expected to vary in accordance with the incidence of dominant lethal-bearing sperm produced by the father.

Several authors have made use of the foregoing argument (STANCATI 1932; BISHOP 1937; WHITING 1937b, 1938; MAXWELL 1938). The results of their experiments have agreed in showing that the numbers of females could be reduced or even eliminated in three-allele crosses of females by males bearing sperm in which dominant lethals had been induced by X-radiation. WHITING's data indicated that at dosages of about 10,000 r units, practically every sperm contained at least one dominant lethal change (number of F_1=.07 per ♀ per day). At lower dosages (2500 r, 5000 r, 7500 r), the numbers of female progeny per day varied inversely with increasing dosage.

The present work was undertaken with the purpose of supplementing the known data by securing larger numbers of progeny and estimates for dosages at smaller intervals. This would allow the construction of a curve showing the relationship between dosage and mortality of females. The author is grateful to DR. P. W. WHITING for proposing the project and for materials and helpful suggestions during the course of the work.

METHODS AND RESULTS

The usual methods used in breeding Habrobracon are rather simple. One or several females are placed in a shell vial with five or six large, active caterpillars of the Mediterranean flour moth, *Ephestia kueniella* Zeller. Within a few hours the females have stung the caterpillars, immobilized them, and fed

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upon their body fluids. Subsequently, the eggs are laid on the surface of the host, and development proceeds. But the yield of adult progeny depends upon a number of variables. The largest numbers of eggs are produced when the mothers are sufficiently young, the caterpillars large, the conditions of temperature fairly high (30°C). Hatchability of the eggs of good stocks under favorable conditions is ninety or more percent. The yield of adult progeny, however, is ordinarily smaller. Wastage may be due partly to competition among the larvae for limited food supply and partly to detachment of eggs and larvae from their hosts. The latter commonly results when caterpillars have crawled to the top or sides of the vial prior to being stung. Losses can be reduced by a rather simple procedure involving the feeding of caterpillars which have already been stung and thus immobilized. The female wasps which are employed to sting the caterpillars are first sterilized by a dosage of 8–10,000 r units of radiation to prevent contamination of the experimental cultures by their eggs. The damage to ovaries and to any stored ova is so severe that oogenesis stops, and all but a few eggs which are laid soon after treatment are inviable.

The above considerations were taken into account because the study of sex ratios required the production of large numbers of progeny. It was already known that well fed, rather young females of good stock would lay 20 or more eggs within a 24-hour period, and it was thought that under suitable conditions, a high proportion of these could be reared to maturity.

The purpose of the first experiments was to secure counts of the female and male adult progeny produced by females which had been out-crossed to irradiated males. Since the plan called for the use of 20 X-ray dosages, it was impossible to do all of the breeding work in a single experiment. Adequate numbers were therefore secured by accumulating the data from seven smaller experiments. The practice adopted was to use only ten dosages at a time and to space them at intervals of 1,000 r beginning either with 500 or 1,000 r. Such staggering of dosages from experiment to experiment would be expected to help in minimizing any systematic errors in the final results. In only one of the seven experiments were data secured for each of the 20 dosages.

The actual technique in a given experiment may now be described. Ten groups of haploid males of stock 1 were X-rayed at intervals of 1000 r units, the lowest dosage in a given experiment being either 500 r or 1000 r, the highest 9500 r or 10,000 r, respectively. The conditions of radiation were kept as constant as possible throughout the entire series of experiments: 200 kv, 25 ma, 21 cm S.T.D., inherent filter equivalent to 4 mm Al. The output ranged from 550 r to 880 r per minute.[2] Within an hour or so following the treatment, each male was mated to a virgin female of wild type stock 33. Females were cultured individually in vials, each of which was supplied with six or seven "pre-stung" caterpillars. Transfer to new vials occurred every two days for a total egg-laying period of ten days. The adult progeny were counted soon after eclosion.

[2] The author is indebted to MRS. EMY SCHILDHAUER, of the AMERICAN ONCOLOGIC HOSPITAL in Philadelphia, for kindly consenting to give the X-ray treatments.

The data and calculations secured as a result of the first experiments are summarized in table 1. Adult progeny were either females or azygous males. No diploid males could arise because there was no possibility of homozygous diploid eggs, since stock 1 bears the sex alleles *xg* and *xh*, and stock 33, *xe* and *xf*. It can be seen from table 1 that among both the mated and unmated controls, the average number of adult progeny per female per day was about nine (8.85 for the unmated controls, and 6.59+2.87 for the mated controls). The

TABLE 1

Data and calculations on adult progeny counts secured in the first experiments.

TREATMENT TO ♂	TOTAL ♀ COUNT	TOTAL ♂ COUNT	TOTAL EGG-LAYING DAYS	MEAN ♀♀ PER DAY	MEAN ♂♂ PER DAY	TOTAL ♀♀ TOTAL PROGENY	MEAN NO. ♀♀ MEAN NO. CONTROL ♀♀
Unmated controls		3966	448		8.85±.30		
Mated controls	3111	1355	472	6.59±.23	2.87±.14	.70	1.00
500r	1402	1177	344	6.29±.18	3.42±.22	.54	.98
1000r	1754	1263	380	4.62±.17	3.32±.10	.58	.70
1500r	1376	1366	334	4.12±.19	4.09±.22	.50	.63
2000r	978	1215	368	2.66±.14	3.28±.16	.45	.40
2500r	667	1163	314	2.12±.12	3.70±.20	.37	.32
3000r	373	1068	244	1.53±.11	4.38±.22	.26	.23
3500r	253	1095	302	.83±.07	3.63±.22	.18	.13
4000r	189	1076	306	.61±.05	3.52±.22	.15	.10
4500r	85	940	344	.25±.03	2.73±.17	.08	.04
5000r	109	1308	344	.31±.06	2.58±.20	.08	.05
5500r	54	977	310	.17±.03	3.15±.21	.05	.03
6000r	32	1093	314	.10±.02	3.48±.27	.03	.02
6500r	18	1192	324	.06±.02	3.77±.22	.02	.02
7000r	14	1166	284	.05±.01	4.11±.26	.01	.01
7500r	14	1055	316	.04±.01	3.34±.21	.01	.01
8000r	5	1387	352	.01±.01	3.94±.17	.00	
8500r	9	991	290	.03±.01	3.42±.22	.01	
9000r	12	1317	334	.04±.01	3.93±.23	.01	
9500r	2	1158	308	.00	3.78±.23	.00	
10,000r	1	1105	292	.00	3.78±.27	.00	

ratio of females to males among the mated controls was 3111:1355 or 2.29:1. Thus 70 percent of the total progeny was female. An inspection of the table shows that the mean number of females per day decreased with increasing dosage in such a way that at roughly 10,000 r virtually no females appeared. The corresponding percentage of females in the total progeny decreased from 70 to 0. But the occurrence of males per day was fairly stable (range=2.58–4.38). This consistency is to be expected, since the number of males is determined by the frequency with which progeny develop from eggs which have not been fertilized and hence cannot be altered in their viability by the sperm. It should be pointed out, however, that there are some significant differences in

the numbers of males per day. This matter will be discussed later in connection with another group of experiments.

The data of table 1 are summarized in a somewhat different way in figure 1, where percentage of female mortality is plotted against dosage in r units. Since the curve becomes nearly asymptotic at 5000 r, it has not been extended to include dosages of higher value. Figure 1 also shows three other curves

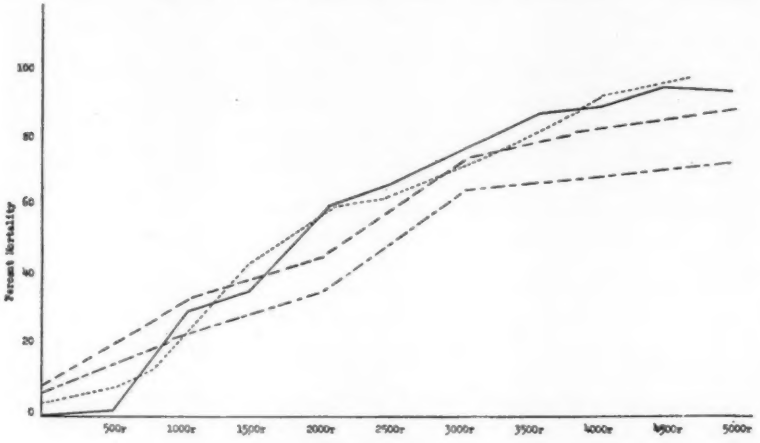

FIGURE 1.—The effects of increasing dosages of X-ray on the incidence of adult wasps, the hatchability of Drosophila eggs, and the incidence of adult Drosophila. (Redrawn from Sonnenblick and from DEMEREC and FANO.)

taken from the work of SONNENBLICK (1940) and of DEMEREC and FANO (1944), and redrawn for purposes of comparison with the curve for Habrobracon. The work on Drosophila has indicated that most of the deaths due to dominant lethality occur prior to the hatching of larvae, and that egg hatchability, therefore, can be used as a criterion for judging the incidence of dominant lethal mutations. An inspection of the curves secured by SONNENBLICK and by DEMEREC and FANO gives an indication of the variability secured at two different times, although the stocks used for the two investigations were nominally the same (Oregon R). This matter of variability of a given strain from time to time in response to similar treatment is discussed at some length by DEMEREC and FANO. Suffice it for our purposes to point out that despite irregularities of this sort, the curves are quite similar to each other and also

to that secured for Habrobracon. Of more pertinent interest, perhaps, is a comparison of the curve for Habrobracon with that which reflects the percentage of adult Drosophila which fail to eclose after irradiation. Here it may be seen that the correspondence in contour, although not exact, is sufficiently close to suggest that similar mechanisms may operate in the production of dominant lethals in the two animals.

Before proceeding with a discussion of the next experiments, it will be necessary to return to a consideration of table 1. The data given there show two points which require further comment. It has already been indicated that among the controls there was an average of about nine progeny per female per day. This value is known to be low in terms of the numbers of eggs which are laid (20 or more per day). It is evident, then, that in spite of the care in feeding, there must still have been a considerable wastage of eggs. Too low a food supply was indicated by the fact that in vials containing the most progeny, the caterpillars were almost entirely consumed. Competition for food was thought to account for the relatively low numbers of males per day among the mated controls (2.87) compared to values secured for most experimental groups—that is, the t value for the significance of the difference between the mean number of males for the mated control group and for the azygous sons of females mated to males treated with 3000 r=4.3. Fluctuations of this sort appeared to be sporadic and not associated with dosage. The question rose as to whether such differences would persist if a technique were devised in which even a greater proportion of the eggs were brought to maturity.

A method which was designed to account for eggs, larvae, and adult progeny was finally developed. Haploid males of stock 1 were X-rayed and out-crossed to virgin females of stock 33. Then each female was placed on a glass plate with one large, "pre-stung" caterpillar, and covered. Transfer to another caterpillar was made every twlve hours for a period of five days. As soon as the eggs and later the larvae had been counted, the larvae were distributed on new caterpillars at the rate of five or fewer per host. Host and larvae were then placed in glass vials and incubated at about 30°C. Counts were made of adult progeny a short time after eclosion.

The data secured from experiments in which the new technique was employed are summarized in table 2. This represents the results of two experiments, one involving the three lowest dosages and conducted in Philadelphia, the other involving the two highest dosages and conducted at Woods Hole.[3] An examination of the table shows that among the mated controls, eggs matured to eclosion at the rate of 14.1 per day (9.4 ♀ +4.7 ♂) in the first experiment and 13.0 per day (8.2 ♀ +4.8 ♂) in the second experiment. Previous experiments had given a comparable value of about 9. Percentages of hatchability showed a steady decline from mated controls to 4500 r (91.5 to 34.3

[3] The author is grateful to MR. L. ROBINSON HYDE of the MARINE BIOLOGICAL LABORATORY, Woods Hole, Massachusetts, for cooperation in giving the X-ray treatment at the two highest dosages.

percent), although the mean numbers of eggs per day remained about constant for the different groups. The percentages of females among the total progeny and the percentage survival of females are closely paralleled by values derived from similar data of the first experiment. The most significant difference between the data from the earlier and present experiments is in the numbers of males per day. The more carefully controlled experiment gave very

TABLE 2

*Data and calculations on egg, larva and adult progeny counts secured in the second experiments.**

TREATMENT TO ♂	MATED CONTROLS	1500 r	3000 r	4500 r	MATED CONTROLS	152,320 r	186,000 r
Total eggs	1447	1584	1296	1150	1194	1618	843
Total larvae	1324	965	531	395	1094	1027	475
Total adult ♀♀	650	377	94	23	516		
Total adult ♂♂	322	385	264	288	304	610	333
Total egg-laying days	69	84	63	62	63	86	49
Mean number of eggs per day	20.97± .90	18.86± .73	20.57± .84	18.55± .89	18.95± 1.00	18.81± .78	17.20± 1.11
Percentage hatchability of eggs	91.5	60.9	41.0	34.3	91.6	63.4	56.3
Mean number of larvae per day	19.19± .73	11.49± .53	8.43± .43	6.37± .45	17.37± .73	11.94± .68	9.69± .71
Mean number of ♀♀ per day	9.42± .54	4.49± .27	1.49± .18	0.37± .07	8.19± .25		
Mean number of ♂♂ per day	4.67± .38	4.58± .32	4.19± .18	4.65± .32	4.83± .36	7.09± .69	6.80± .61
Total ♀♀	.67*	.49	.26	.07	.63		
Total progeny	(.70)	(.50)	(.26)	(.08)	(.70)		
Mean ♀♀	1.00	.48	.16	.04	1.00		
Mean control ♀♀	(1.00)	(.63)	(.23)	(.04)	(1.00)		

* Values in parentheses copied from table 1, for comparison.

uniform values (4.19 to 4.67 per day) for all groups. This is the result expected but not satisfactorily attained in the earlier experiments.

Since the method of the second experiments was somewhat more satisfactory than that of the first, it was decided to use it under conditions of very high dosage. The question has risen from time to time as to whether or not sperm could be inactivated by high X-ray dosage and thus prevented from entering into the fertilization process. In Habrobracon an egg which receives no sperm is expected to develop into an azygous male. If this occurred in an appreciable number of cases, the mean number of males per day should be increased in comparison to the number secured when no treatment was given, and when lower dosages were used. MAXWELL (1938) has presented evidence to show that inactivation of sperm does occur at dosages of 142–143,000 r. Her data

indicated 3.12 males per day for unmated controls, 1.05 for mated controls, and 2.01 for out-crosses of virgin females bred to irradiated males.

The experiments with high dosage were carried out at Woods Hole where high intensities are delivered by two water-cooled G.E. (XTP) tubes in opposed position. The output at 182 kv, 25 ma, and distance equal to 9.5 cm, was estimated at 4480 r per minute. At a distance of 14.4 cm, the output was 2400 r. Two experiments were performed. In the first, a total dosage of 152,320 r was delivered at a distance of 9.5 cm; in the second, 168,000 r at 14.4 cm.

Before proceeding with a discussion of the results obtained for high dosages, an unexpected observation should be mentioned. In the first and all later attempts to give treatments at high dosages, it was noticed that the wasps began to look "sluggish" after having received about 125,000 r, although precautions had been taken to eliminate effects due to heat (by fans and water coolers). If the treatment was stopped at this dosage and the wasps returned to glass vials, a kind of recovery occurred. That is, at the end of a few hours or less, a vigorous, normal activity was restored, and observed matings were rather easily secured. At somewhat higher dosages, roughly 150,000 r to 170,000 r, the "sluggishness" was more marked but the recovery good. At still higher dosages, 249,000 r and 300,000 r, recovery did not occur, although slight twitchings could be seen immediately after the treatment. The wasps which received 152,320 r and 168,000 r were nearly immobile after treatment. But within about four hours, they had recovered sufficiently to permit observed matings, and no deaths occurred within the following two days. Tests for sperm inactivation were made by counting the eggs, larvae, and adult progeny produced by the impregnated females throughout a period of eight egg-laying days. Control counts were made from comparable crosses of virgins of stock 33 by untreated males of stock 1. The data are summarized in table 2.

A comparison of the data for the mated controls with those for the experimental groups readily shows a significant difference between the means of adult male progeny ($t = 2.9$ for the difference between $4.83 \pm .36$ and $7.09 \pm .69$, and $t = 2.8$ for the difference between $4.83 \pm .36$ and $6.80 \pm .61$). The relatively large values for the adult progeny were foreshadowed by a high rate of hatchability among the eggs (63.4 percent and 56.3 percent, as compared to 34.3 percent for a dosage of only 4500 r). In fact, the rate of hatchability for very high dosages was about the same as that for only 1500 r. Such percentages offer strong evidence that the high dosage of radiation had in some way interfered with the fertilization process.

The data for hatchability may be regarded from another point-of-view. It is known from the control data for the two highest dosages that the adult male progeny constituted 37 percent of the total. This may be interpreted to mean that of all eggs laid, 63 percent were potentially female and 37 percent male. In control experiments, about 92 percent of all eggs hatch. Thus the percentage of eggs which would hatch as male larvae should be $.92 \times .37$. At very high dosages where no female offspring are produced, this value multiplied by the number of eggs laid should give the total number of larvae expected, provided

there were no interference with the fertilization process. If sperm were inactivated, the calculated value would be too low, because unfertilized eggs are expected to be male.

The expected numbers of larvae for low dosages can also be calculated. Here the number of male larvae should equal the number of eggs laid×.33 ×.92. The number of female larvae should be equivalent to: number of eggs laid×.67×.92×R/2.02, where .67 represents the percentage of fertilized eggs, .92 the normal hatchability rate, 2.02/1 the ratio of adult females to males among controls, and R the ratio of adult females to males at a given dosage. Calculations for the expected numbers of larvae are presented in table 3.

TABLE 3

A comparison of larvae observed and expected when treatment of the sperm is assumed to have no effect on the fertilizing capacity.

TREATMENT TO ♂	OBSERVED LARVAE	PERCENTAGE OF EGGS HATCHED	EXPECTED LARVAE	PERCENTAGE OF EGGS EXPECTED TO HATCH
1500r	965	61	954	60
3000r	531	41	553	43
4500r	395	34	381	33
152,320r	1027	63	551	34
168,000r	475	56	287	34

Here it may be seen that there is only a slight discrepancy between the numbers of observed and expected larvae, for the three lowest dosages. These differences may readily be accounted for on the basis of errors of sampling. But at the two highest dosages, the differences are conspicuous. Sperm must have failed to enter the eggs in an appreciable number of cases (about 40 percent). One can only speculate as to whether the effect on the sperm was direct or by way of fluids in which they were suspended.

SUMMARY

Haploid males of the parisitic wasp, Habrobracon, were X-rayed with dosages ranging from 500 r to 10,000 r units and crossed to untreated females of an unrelated stock. Diploid females are expected to rise from the fertilized eggs, haploid males from the unfertilized ones. The number of females per day decreased with increasing dosage. Dosage of roughly 10,000 r induced at least one dominant lethal in every sperm. The number of males per day remained relatively constant. Mortality of females when plotted against dosage gave a non-linear relationship.

Special techniques which allowed the counting of eggs and larvae, as well as adult progeny, indicated a decrease in hatchability and adult female progeny, a highly uniform number of males per day for dosages up to 4500 r. But the number of males for very high dosages, 152,320–168,000 r, showed a significant increase. This is interpreted to mean that a fairly high proportion of sperm (about 40 percent) were inactivated in some way.

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